Epidemiological studies of downy mildew of oilseed poppy

by

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This thesis contains no material that has been accepted for the award of any other higher degree or diploma in any University, and to the best of my knowledge contains no paraphrase of material written or published by any other person except where due reference is made in the text.

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Abstract

Downy mildew is a major limiting factor of oilseed poppy production in Tasmania. However, little knowledge of the epidemiology of the disease currently exists. The objectives of this project were to taxonomically identify the downy mildew pathogen, characterise the spatiotemporal development of epidemics, analyse the effect of weather variables on epidemic development, and identify the means of overwintering by the pathogen.

Phylogenetic analysis of the ribosomal DNA region, including the internal transcribed spacer regions and the 5.8S gene, indicated the downy mildew pathogen is *Peronospora cristata*, not *P. arborescens* as previously reported. Conidium dimensions were unable to distinguish between the two species.

Under favourable disease conditions epidemics can develop rapidly, with disease incidence increasing in a field trial from 0.2 % to 100 % over a 40 day period during the 2001/2002 growing season. Epidemics were spatially aggregated after the onset of canopy closure, while the spatial pattern at an individual time was significantly associated with the spatial pattern that occurred 10 days prior. Under spatially aggregated plant densities the local area under disease progress curves (AUDPC) of both disease incidence and severity was positively correlated and spatially associated with high plant densities. These results indicate that downy mildew epidemics were dominated by secondary spread, from low levels of primary inoculum.

Downy mildew infection was observed to decrease alkaloid content, but not capsule dry matter yield. Alkaloid content of capsules was significantly spatially dissociated

with the local AUDPC of both disease incidence and severity in both the 2000/2001 and 2001/2002 growing seasons. Capsule dry matter yield in poppy crops was not consistently correlated or spatially associated with the local AUDPC of either disease incidence, or severity over both of these seasons.

The forecaster model, DOWNCAST, developed for the prediction of epidemics of onion downy mildew (*P. destructor*), provided moderate prediction of sporulation and infection events during poppy downy mildew epidemics. Accuracy of prediction by the model was increased by increasing the critical limit for sporulation inhibition by nighttime rainfall to 3 mm, and decreasing the leaf wetness critical limit for infection when using Watchdog® 450 dataloggers from 7.5 to 4.5.

The principle means of overwintering by the downy mildew pathogen appears to be via the 'green bridge' provided by regrowth poppy plants, and other *Papaver* spp. Downy mildew oospores were found associated with the residues of poppy crops and survived at least 26 months burial in uncultivated soil. *Peronospora cristata* oospores were also detected in association with the seed of poppy, by a seed washing technique and molecular detection using the polymerase chain reaction. However, no evidence for primary infection resulting from oospores was recorded.

1. Introduction

Commercial oilseed poppy (*Papaver somniferum* L.) production in Australia is situated in the southern most state, Tasmania (Appendix I). In 2000, the poppy industry included approximately 1400 growers, with a total area of 20,645 ha (International Narcotics Control Board 2002) with the majority situated in the north-west of the state. This equated to an export value of some US$100 million.

Oilseed poppy is grown in Tasmania for the production of the alkaloids morphine, codeine and thebaine. These are harvested as part of the dry capsule of the poppy plant and then processed into a variety of pharmaceutical products, predominantly for export markets. A by-product of alkaloid production, poppy seed, is sterilised and marketed for culinary consumption.

The predominant disease limiting poppy production in Tasmania at present is downy mildew (A. J. Fist, Tasmanian Alkaloids. Pty Ltd., *personal communication*; P. J. Cotterill, GlaxoSmithKline, *personal communication*). Downy mildew is one of the most destructive diseases of opium poppy in the world (Kapoor 1995). Since its first record in 1996 (Cotterill & Pascoe 1998), the disease has become prevalent in Tasmania, affecting most crops each season. Typically, symptoms of the disease are dark brown 'oilspot' lesions on leaves that increase in size over time until they became angular when limited by the veins of the host plant. Following favourable environmental conditions, a white 'downy' growth of conidiophores is formed on the abaxial surface of leaves. In wet and humid conditions, downy mildew can spread rapidly throughout the crop, reducing the yield of opiates (P. J. Cotterill, *personal communication*). In Europe, downy mildew has been responsible for the complete

loss of crops (Yossifovitch 1929). The pathogen responsible for downy mildew of oilseed poppy in Tasmania has been recorded as the oomycete *Peronospora arborescens* (Berk.) Casp. (Cotterill & Pascoe 1998). However, taxonomy of *Peronospora* species infecting members of the *Papaver* plant genus is based on conidium dimensions (Reid 1969), which can vary with environmental conditions (Hall 1996), and is therefore ambiguous.

Due to its relatively recent discovery, little research has been conducted into the epidemiology of poppy downy mildew in Tasmania. The lack of epidemiological knowledge of disease development, and how this is affected by both crop and climatic factors is a potential limiting factor to the development of management strategies for the control of poppy downy mildew in Tasmania. Similarly there is little information available on the means by which the downy mildew pathogen overwinters between crops. Such information may allow the development of improved control strategies for the disease.

The aims of this project were:

1. To confirm the taxonomic identity of the poppy downy mildew pathogen, and determine the intraspecific variation within the pathogen population in Tasmania.
2. Develop a molecular test for the detection of poppy downy mildew.
3. To characterise the spatiotemporal development of epidemics of poppy downy mildew and its relationship to crop characteristics.
4. Validate the forecaster model, DOWNCAST, for the prediction of poppy downy mildew epidemics based on measured weather parameters.
5. Evaluate the potential means by which the poppy downy mildew pathogen overwinters.

2. Literature review

2.1. Oilseed poppy

2.1.1. Botany

The oilseed (or opium) poppy, *Papaver somniferum* L., is a member of the family Papaveraceae, containing 42 genera, which is known for the number and variety of alkaloids produced by its members (Kapoor 1995). *Papaver somniferum* is of particular interest for agricultural production due to its ability to produce several medicinally valuable alkaloids, including morphine, codeine and thebaine (Williams & Ellis 1989). These alkaloids are used in the synthesis of a wide variety of pharmaceutical products.

A native of Mediterranean regions, *Pap. somniferum* is an annual herb producing thick roots and a thick stem able to grow to 1.5 m in height (Curtis 1993). The leaves of mature plants are lobed, alternate, sessile, and clasp the stem, with some branching of the stem possible (Fig. 2.1A). Leaf size reduces up the height of the plant. Developing flower buds form a characteristic hook downwards (Fig. 2.2), but become upright upon flower burst (Fig. 2.2). Flowers are single, perigynous and terminal, with two sepals that are lost on flower opening, and four petals (Fig 2.1B). Petal colour can vary in colour from white to lilac, with a darker base to red (Fig. 2.3). Petals typically begin to drop within two days of flower opening (Kapoor 1995). The fruit of the plant is a capsule, more or less spherical in shape, up to 40 mm in diameter, capped with a persistent stigmatal disk (Fig. 2.1C). Cross-pollination is common, allowing the formation of hybrids between the different varieties (Kapoor 1995). The morphological characteristics in the cultivated poppy can also vary dependent on breeding (Kapoor 1995).

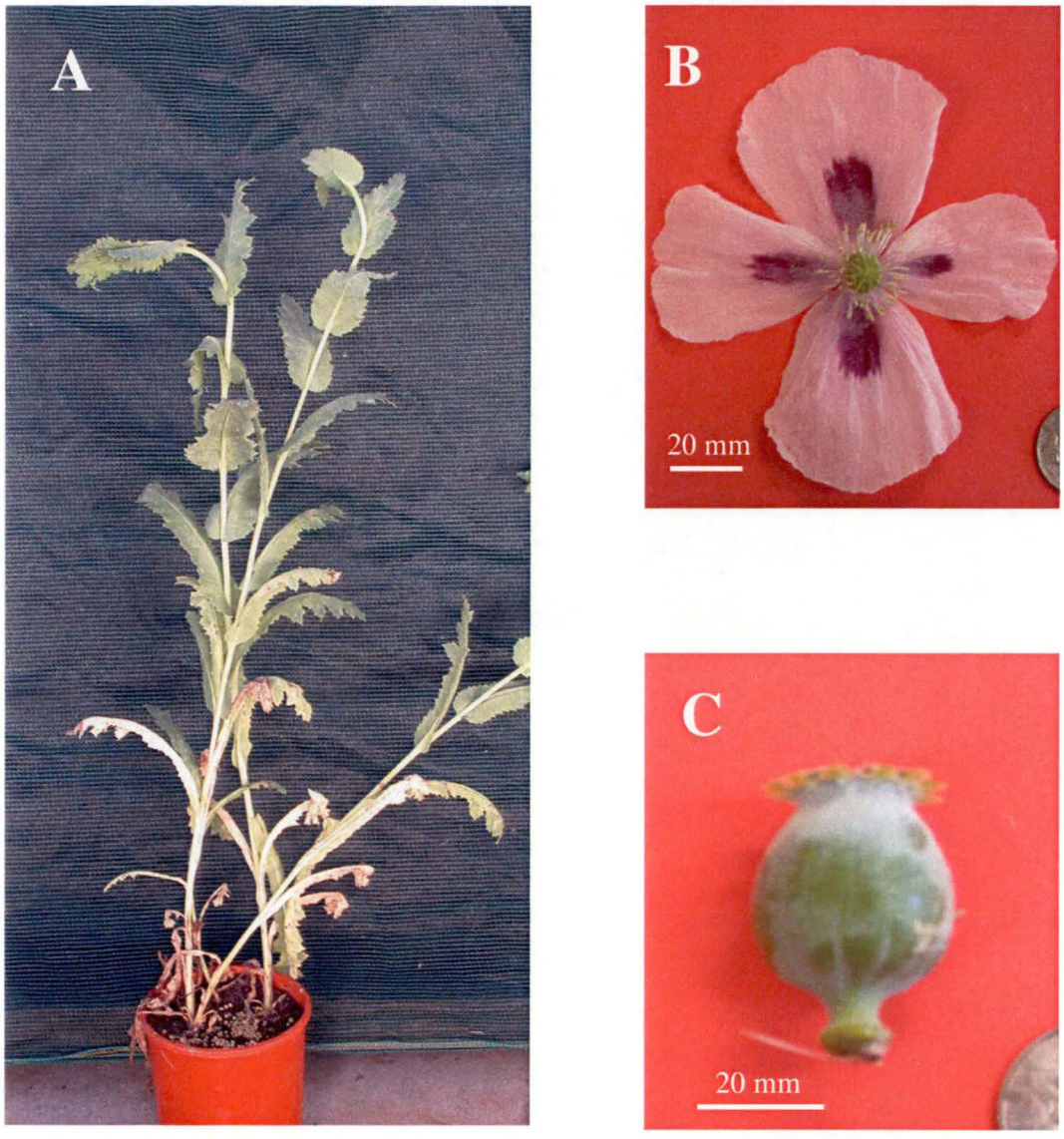


Fig. 2.1 Photos of the morphology of *Papaver somniferum* L.; A) whole plant; B) flower; C) capsule.

Fig. 2.2 Field of *Papaver somniferum* L. showing the three phases of reproductive development; A) hooked bud; B) flowers; C) capsules.

Fig. 2.3 Field of *Papaver somniferum* L. showing the variety of petal colours possible (Photo courtesy of Dr. David Russell, University of Tasmania).

2.1.2. History and current production

Oilseed poppy is one of the oldest known medicinal crops (Kapoor 1995). References can be found to the cultivation of the crop from as far back as the peak of the ancient Sumerian civilisation (Bernath 1998a; Kapoor 1995). From this evidence it is assumed that the plant originated in middle Asia, including modern day Iraq, which was the site of Sumeria (Bernath 1998a; Kapoor 1995). From here, the spread of the poppy can be followed through the conquest of Sumeria by the Assyrians in the 6th Century BC (Kapoor 1995) to its spread to the Greeks, Romans and Egyptians (Bernath 1998a; Kapoor 1995). The spread of the crop from the Mediterranean into the rest of Europe is credited to the Roman Empire (Bernath 1998a), while its spread east into Asia is attributed to the traders of the Arab Empire from the 7th Century AD onwards (Kapoor 1995).

The traditional uses of the crop revolve around the use of the latex of poppy capsules, called opium, derived from the Greek word 'opos', meaning poppy juice (Husain & Sharma 1983; Kapoor 1995). Opium was principally used in ancient times for pain relief, religious ceremony, sleep-inducement (Kapoor 1995) and in some cases euthanasia (Bernath 1998a). In more recent times, poppies have been cultivated in eastern Europe for seed production, both for culinary purposes and stock feed (Bernath 1998a).

The opium poppy is unique amongst the plant kingdom, being the only plant able to naturally produce the alkaloid morphine and other important alkaloids such as codeine and thebaine (Anonymous 1999; Bernath 1998b). Morphine, a term derived from 'Morpheus', the Greek god of sleep (Husain & Sharma 1983), is an important

pain relief drug used in modern medicine, while many other medicines are produced from various other opiate alkaloids (Anonymous 1999). The invention in 1928, of a processing technique for poppy straw (capsules and some stem material) for the extraction of alkaloids by the Hungarian pharmacist Janos Kabay, modernised poppy cultivation (Bernath 1998a). The process has made poppy production for alkaloid extraction an economically viable option in western countries, along with the collection of poppy seeds for sale (Bernath 1998a).

Licit cultivation of oilseed poppy is now controlled by United Nations mandate, which attempts to limit the levels of cultivation by production centres and the world as a whole, to match levels of demand (International Narcotics Control Board 2002). In 2000, 384.3 tons of morphine equivalent opiate raw materials were produced worldwide (International Narcotics Control Board 2002). The major producers in 2000 were India (38 % of total production), Australia (29 %), France (10 %), Turkey (9.3 %) and Spain (9.1 %). Indian opiate production is still conducted using traditional, labour intensive methods of opium collection, with India producing in excess of the 99 % of the world's licit opium in 2000 (International Narcotics Control Board 2002). Australia, France, Turkey and Spain all harvest poppy straw using the methods of Kabay to process straw into concentrated poppy straw (International Narcotics Control Board). In addition to morphine production, thebaine rich poppy varieties are now cultivated in Australia (79 % of total world production) and France (21 %), with a total world production of 49.1 tons of thebaine equivalent (International Narcotics Control Board 2002). Low levels of poppy cultivation also occur in eastern Europe for seeds for culinary consumption.

2.1.3. Cultivation in Australia

Poppy production in Australia has occurred on a small scale since the 19th Century. At this time, cropping was for the production of opium for use in local medicines such as laudanum (Laughlin *et al.* 1998). During the Second World War trial work and semi-commercial production was undertaken based upon straw processing, due to the shortfall in morphine imports from the Northern Hemisphere (Laughlin *et al.* 1998). After the end of the Second World War, local production ceased and Australia again relied on importation for morphine supplies. In 1961, fluctuations in supply from the Northern Hemisphere led to trial work being undertaken in Tasmania and in 1970 large scale production based on straw processing began in the state (Anonymous 1999). Production in Australia is currently limited to Tasmania due to state ministerial agreement based on the security provided by the isolated nature of the state (Laughlin *et al.* 1998). The Tasmanian industry is controlled by two processing companies, GlaxoSmithKline and Tasmanian Alkaloids Pty Ltd. (a subsidiary of Johnson & Johnson). In 2000, approximately 1400 growers, with a total area 20,645 ha cultivated poppies in Tasmania (International Narcotics Control Board 2002) with the majority situated in the North-West of the state.

Poppy crops in Tasmania are sown as seed in the late-winter/early-spring months of August and September. Poppies prefer fine seed beds prepared in sandy loam soils of neutral to slightly alkaline pH. Seeds are usually band sown with fertiliser tailored to soil requirements. Seedling emergence usually takes place within one to two weeks (Fig. 2.4).

Fig. 2.4 Oilseed poppy seedlings, indicated by arrows, 21 days after sowing.

Early in their development the competitive ability of poppy seedlings is poor (Kapoor 1995; Nemeth 1998). This necessitates the use of chemical herbicides to control weeds early in crop development, at the four to six leaf stage.

In addition to the chemical fertilisers applied at sowing, nitrogen is top dressed to crops following the onset of stem elongation.

Poppy production in Tasmania is conducted under both dryland and irrigated conditions, using overhead gun and centre pivot irrigators. The critical times of irrigation to maximise yields are bud hooking, later flowering and two weeks after the end of flowering (Chung 1987).

Poppies are characteristically long day plants requiring 16 hour day lengths to induce flowering (Wang *et al.* 1997). Under Tasmanian conditions this typically results in flowering in December; i.e., early summer (Fig. 2.5). Following capsule maturation, crops are allowed to dry off until the moisture content of capsules has reached 12 % (Anonymous 1999) before mechanical harvesting (Fig. 2.6) occurs. Harvested capsules are processed into CPS for export or undergo further processing for the production of active pharmaceutical ingredients (International Narcotics Control Board 2002). Seeds from capsules are cleaned and sold for culinary purposes.

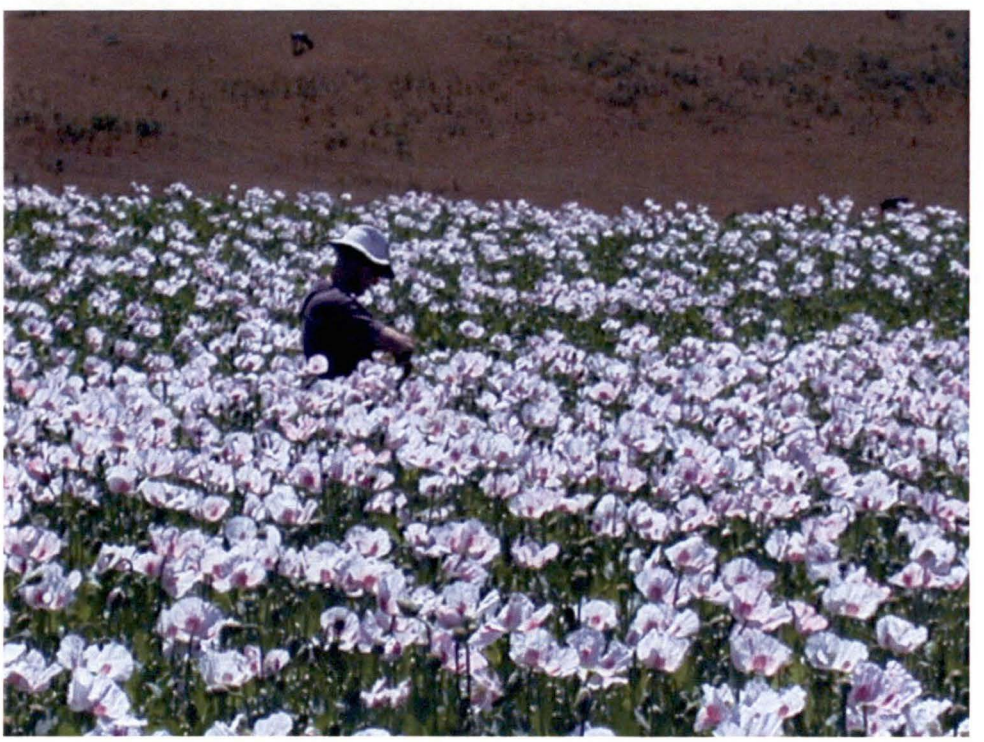


Fig. 2.5 Oilseed poppy field in full flower.

Fig. 2.6 Mechanical harvesting of a dried oilseed poppy crop.

2.1.4. Major pathogens

Oilseed poppy is subject to a number of diseases caused by bacterial, viral and fungal pathogens. Bacterial infections by species such as *Pseudomonas solanacearum* and *Erwinia carotovora*, can cause cell lysis and tissue breakdown (Janardhanan & Husain 1983). In Tasmania, bacterial infections cause only occasional problems, typically affecting only single plants (Dennis 1998). Several viral pathogens have been reported to infect oilseed poppy (Janardhanan & Husain 1983; Kapoor 1995), although none are significant disease concerns. In Tasmania, tomato spotted wilt tospovirus has been reported to caused latent infections of poppy in glasshouse trials (Wilson 1999), but no reports of viral pathogens in the field have been published. However, unpublished surveys have detected an unknown potyvirus, affecting poppy in the field (C. R. Wilson, University of Tasmania, *personnal communication*).

Based on current knowledge, the major diseases of poppy are all caused by fungal and oomycete pathogens.

Poppies are susceptible to a number of damping off and root rot pathogens. Damping off of seedlings has been reportedly caused by *Fusarium solani* in India (Kishore *et al.* 1985). *Pythium* spp. are known to be responsible for damping off in Australia (Angell 1950). Root rots of older plants have been reported as caused by the pathogens *Macrophomina phaseolina* and *Fusarium* spp. (Kapoor 1995).

A variety of fungal pathogens are known to attack the capsules of oilseed poppy. These include *Dendryphion penicillatum* (Farr *et al.* 2000; Janardhanan & Husain 1983), *Macrosporium papaveris*, *Botrytis cinerea* (Kapoor 1995), *Stemphylium* spp., *Alternaria* spp., and *Cladosporium* spp. (Dennis 1998). Fungal infections of this kind can significantly reduce the alkaloid content (Dennis 1998).

In Tasmania, yield reductions can also result from stem infections by the pathogen *Sclerotinia sclerotiorum* (Dennis 1998; Laughlin *et al.* 1998), which weakens stems and increases the occurrence of lodging in mature crops (Dennis 1998; Laughlin *et al.* 1998). Lodging reduces the amount of capsules able to be harvested mechanically.

Several fungal pathogens are also known to affect poppy foliage. Kishore *et al.* (1985) reported *Alternaria alternata* and *Dreschlera spicifera* as foliar pathogens in India. Leaf smut, which can be a problem in temperate regions, is caused by the pathogen *Entyloma fuscum* (Dennis 1998; Laughlin *et al.* 1998). Severe epidemics

of leaf smut can cause early defoliation of crops resulting in reduced yields (Dennis 1998; Laughlin *et al.* 1998).

Poppy fire is a disease that attacks both the leaves and capsules of oilseed poppy, causing losses sufficient to warrant disease control in many situations. Recently, it has been shown that the poppy fire is caused by either of two pathogens *Pleospora papaveracea* and *Dendryphion penicillatum* (Farr *et al.* 2000). It was previously thought that *Pl. papaveracea* was the teleomorph of *D. pencillatum*, but studies by Farr *et al.* (2000) have disproven that link. It has been shown that *Pl. papaveracea* is the more virulent of the two species (Farr *et al.* 2000).

Powdery mildew is another disease known to cause significant reduction in yield. Powdery mildew is reportedly caused by *Eyrsiphe polygoni* (Audichya & Thakore 2000), *Oidiopsis* sp. (Kapoor 1995) and *Oidium* sp. (Dennis 1998; Kapoor 1995). In Tasmania, powdery mildew normally develops late in the growing season and is considered unlikely to cause yield reductions (Dennis 1998).

The most destructive disease of oilseed poppy is downy mildew (Kapoor 1995; Rathore *et al.* 1986). Total crop losses have been reported due to downy mildew epidemics in Europe (Yossifovitch 1928). The disease is also known to be a significant problem in Iran (Scharif 1970) and India (Kothari & Prasad 1970). It has also been reported in Africa, South America (Francis 1981), and North America (Tewari & Skoropad 1981). In 1996, downy mildew was first recorded in Tasmanian poppy crops (Cotterill & Pascoe 1998). The disease had previously been observed in Tasmania on wild poppy species (*Papaver* spp.) surrounding commercial crops in 1995 (Cotterill & Pascoe 1998). How the disease came to enter Tasmania is

unknown. Downy mildew of opium poppy has been reported to be caused by the oomycete species *Peronospora arborescens* (Berk.) Casp. (Behr 1956; Cotterill & Pascoe 1998; Francis 1981), and represents a significant risk to the Tasmanian poppy industry. Since its initial discovery, downy mildew has become prevalent in the majority of crops statewide.

2.2. Downy mildew of poppy

2.2.1. Symptoms

The disease symptoms of downy mildew infection of poppy plants are similar to downy mildew infections on other plants. Typically signs of infection are white to pale-violet hyphal growth on the undersides of leaves. In addition, dirty-brown coloured necrotic lesions ('oilspots') on the upper leaf surface are symptomatic of infection (Behr 1956; Kothari & Prasad 1970; Wheeler 1981; Yossifovitch 1929). These lesions are often surrounded by chlorotic rings and become angular in shape when limited by the veins of the host plant (Behr 1956; Kothari & Prasad 1970).

The type of symptoms expressed is often dependent upon the plant age at the time of infection. It is generally accepted that the most susceptible stage of poppy growth to downy mildew infection is early in the development of the seedling. Observations indicate that the highest levels of crop infection occur when inoculum is introduced at the seedling stage (Behr 1956; Kothari & Prasad 1970). When infected at the seedling stage opium poppy plants tend to become stunted and die rapidly (Behr 1956; Yossifovitch 1929). Seedlings that survive infection at this age often exhibit leaf and bud deformations (Kothari & Prasad 1970; Yossifovitch 1929). At later growth stages the symptoms of infection are principally those described above, affecting mainly the oldest leaves (Behr 1956; Nigam *et al.* 1989; Yossifovitch 1929). Leaves may become deformed with time and sometimes die (Behr 1956; Kothari & Prasad 1970). These observations indicate that early infections of poppy crops tend to be much more damaging than later infection.

Downy mildew in other crops also tends to be more severe on young plants. Soybean (*Glycine max* (L.) Merr.) seedlings grown under 16 hour photoperiods and held at 20 °C during the day and 10 °C at night were found to have decreased severity of disease caused by *Peronospora manshurica* (Naum.) Syd. ex Gaum. with increasing plant ages from 5 to 24 days after sowing (Wyllie & Williams 1965). As disease severity decreased the number of necrotic lesions was found to increase (Wyllie & Williams 1965). However, with the increase in lesion number a corresponding decrease in lesion size was also observed (McKenzie & Wyllie 1971; Wyllie & Williams 1965). The relationship between disease severity and plant age was not linear. The critical plant age for infection was 15 days, beyond which point disease severity significantly decreased and the average number of lesions significantly increased (Wyllie & Williams 1965). When temperatures were increased to day temperatures of 25 °C and night temperatures of 15 °C a similar pattern was observed, although the critical age beyond which disease severity decreased was at 11 days. A further increase in temperatures to 30 °C day and 20 °C night decreased the critical age to eight days. The authors noted that the physiological stages of 11 days old seedlings grown at 25 °C/15 °C (day/night temperatures) were roughly equivalent to 15 days old seedlings grown at 20 °C/10 °C (Wyllie & Williams 1965). No direct comparison of physiological stage was made for seedlings grown at 30 °C/20 °C.

Mence and Pegg (1971) showed that the susceptibility of pea (*Pisum sativa* L.) plants to infection by *Peronospora viciae* (Berk.) Casp. also decreased with age. Resistance to infection increased with leaf age until senescence, at which point resistance decreased (Mence & Pegg 1971). If these findings hold true for poppy downy mildew, as has been suggested by Nigam *et al.* (1989), this may well explain

why the symptoms of infection are principally seen on the older leaves in mature crops. They would also explain why a more severe expression of infection, including plant death is observed in young seedlings, while less severe localised necrotic lesions appear more common on older plants.

2.2.2. Taxonomy

Taxonomy of the genus *Peronospora*

The causal organisms of the downy mildew group of diseases are all members of the family Peronosporaceae, which is part of the order Peronosporales in the class Oomycota (Lebeda & Schwinn 1994; Shaw 1981).

The oomycetes are distinguishable from other fungi by the presence of elongated mycelium without septate divisions, the sexual production of oospores and asexual production by either sporangia leading to zoospores, or conidia (Agrios 1988; Brown 1997). The mycelial walls of oomycetes are (unlike other fungi) primarily composed of cellulose, β-glucan and little chitin (Brown 1997).

The Peronosporales are obligate parasites that form intercellular mycelial networks within the host plant (Brown 1997). These mycelial networks also include well-developed haustoria (Brown 1997).

The Peronosporaceae consist of those fungi that form the characteristic foliage blights of downy mildews on non-graminaceous host plants (Agrios 1988; Brown 1997). They are also identified by producing sporangiophores which can be differentiated from mycelia based on their distinct branching (Agrios 1988). Unlike

the other family within the Peronosporales, the Albuginaceae, the Peronosporaceae form individual sporangia on the ends of sporangiophores, not sporangial chains (Brown 1997).

The genus *Peronospora* is based upon a variation in the sporangial stage of reproduction. Asexual reproduction in *Peronospora* does not involve the production of motile zoospores. Instead, *Peronospora* spp. produce sporangia which germinate directly to produce aseptate germ tubes (Lebeda & Schwinn 1994; Shaw 1981). These germ tubes then penetrate the host plant to initiate infection. To differentiate between the two sporangial forms, those sporangia that germinate directly via germ tubes are usually termed 'conidia', and their sporangiophores, 'conidiophores'. The genus is distinguished from the genus *Bremia*, which also forms conidia, by the morphology of the conidiophore tips (Crute & Dixon 1981). The *Peronospora* form fine tapered terminal branches on their conidiophores (Shaw 1981), while the genus *Bremia* produce their conidia on flattened disk shaped tips with bordering sterigmata (Crute & Dixon 1981).

Speciation within the genus *Peronospora* is problematic. Initial species differentiation was based on one of two systems. Morphological differentiation of species in the genus *Peronospora* is based upon the recorded sizes of conidia, conidiophores and oospores. However, this has proved unsatisfactory due to the wide variation observed among *Peronospora* species isolates from the same host species (Clayton & Stevenson 1943; Stuteville 1981; Yerkes & Shaw 1959; Yossifovitch 1929). For example, Behr (1956) reported the average diameter of oospores of *P. arborescens* to be 30.4 µm, Maiti and Chattopadhyay (1986) reported oospores of 22 to 23 µm in size, while Yossifovitch (1929) recorded oospore

diameters between 23 and 39 µm. Yerkes and Shaw (1959) noted that the size of the reproductive organs of many *Peronospora* species is affected by a range of environmental factors, including temperature, moisture levels and climate. Likewise, Behr (1956) observed that the conidia of *P. arborescens* could be grouped based on geographic distribution of the isolates in question. This would indicate that the environmental conditions during formation play a significant role in the size of conidia upon maturation. Due to these observations, some authors have argued that the size variation observed for the reproductive organs is too great to be the sole determinant of species in the genus (Clayton & Stevenson 1943; Hall 1996; Shaw 1981; Yerkes & Shaw 1959).

The second method by which species have been differentiated in the past is based upon the close relationship between the obligate parasite and the host plant. The close relationship is assumed to have led to very narrow host ranges for *Peronospora* species and therefore the host ranges of isolates were used to determine species (Clayton & Stevenson 1943; Yerkes & Shaw 1959). Some authors consider differentiation based on host range to be unsatisfactory as it has created what is observed to be an artificially high numbers of species (Shaw 1981; Yerkes & Shaw 1959), partially due to the existence of individual races within fungal species. This is evident in the species *P. manshurica* where 31 races have been described (Dunleavy 1977) based upon their abilities to infect different cultivars of soybean (Dunleavy 1971; Dunleavy 1977). Degrees of physiological specialisation have also been observed in the species *Peronospora parasitica* (Pers. ex. Fr.) Fr. that infect a number of species in the family Brassicaceae (Channon 1981; Yerkes & Shaw 1959), and *Peronospora tabacina* Adam which causes blue mould of tobacco (*Nicotiana tabacum* L.; Reuveni *et al.* 1988).

Yerkes and Shaw (1959) offered a compromise between these two methods. Under their method, the plant host family was first used to differentiate species. Secondly, if the observed range of isolate reproductive organ sizes obtained from a single host species overlapped with the range obtained from a second host species then the two samples in question should be considered the same species. If there was no overlap then these samples were assigned to different species. Shaw (1981) proposed another method of distinguishing species based on the morphology of oomycete organs inside the host plant. Shaw (1981) considered that the mode and shape of branching of the conidiophores were more suitable determinants of species due to the reduced variation of these features among isolates.

A more accurate definition of species may be obtained through combining phylogenetic analyses of the molecular characters with morphological characters (Hall 1996). Such techniques have already been used to determine phylogenies for the Peronosporales as a group (Cooke & Duncan 1997) and to differentiate between species in the genus *Phytophthora* (Cooke & Duncan 1997; Möller *et al.* 1993; Panabieres *et al.* 1989). Previous studies have shown the internal transcribed spacer (ITS) regions of ribosomal RNA genes (rDNA) to be useful for the differentiation of downy mildew species and related organisms at the species level (Constantinescu & Fatehi 2002; Cooke *et al.* 2000a; Crawford *et al.* 1996). Molecular probes have also been used to confirm the species of isolates of *P. tabacina* (Wiglesworth *et al.* 1994) and *Peronospora sparsa* Berk. (Lindqvist *et al.* 1998). The author has been unable to find published reference to any studies of this type undertaken specifically on *Peronospora* on *Papaver* spp.

Taxonomy of *Peronospora* spp. on *Papaver* spp.

Due to the lack of molecular studies, the taxonomy of *Peronospora* spp. on *Papaver* spp. is still principally based upon the morphology and host specificity of fungal isolates. Five species from within the genus *Peronospora* have been recorded as infecting members of the plant genus *Papaver*, *P. arborescens*, *P. argemones* Gaum., *P. cristata* Tranz., *P. grisea* Ung. var. *minor* Casp., and *P. papaveris-pilosi* Viennot-Bourgin (Constantinescu 1991). However, the status of these species is complicated. *Peronospora papaveris-pilosi* is considered an invalid record, whilst *P. grisea* var. *minor* is considered conspecific with *P. arborescens* (Constantinescu 1991). Conspecificity between *P. argemones* and *P. cristata* has also been argued based on the similarity of published conidium dimensions, and the older *P. cristata* taxon preferred (Reid 1969). In the same study, *P. cristata* was differentiated from *P. arborescens* based on the latter's smaller average conidium dimensions on *Meconopsis* spp. (Reid 1969). The culmination of these findings suggests that two different species of *Peronospora* are able to infect the plant genus *Papaver*, *P. arborescens* and *P. cristata.*

The conidia of *P. arborescens* are hyaline or light violet in colour (Maiti & Chattopadhyay 1986), and of two differing shapes, either elliptical or ovoid (Behr 1956; Yossifovitch 1929). The size of conidia also varies, even when recorded from the same host species, *Pap. somniferum.* Yossifovitch (1929) recorded the size of the oval form of conidia to be 13 to 26 μm in length and 11 to 19.5 μm in width, with average values of 18.7 and 16.3 μm respectively. The elliptical form was recorded as being 16 to 29 μm in length and 14 to 21 μm in width, with respective averages of 23.3 and 16.3 μm. Behr (1956) observed narrower ranges, with the length of conidia varying from 19.8 to 24.2 μm and the width of conidia ranging from 18.0 to 19.4 μm.

Reid (1969) reported the size of British isolates of *P. arborescens* to range from 11.4 to 27.9 µm in length and 8.5 to 21.7 µm in width. Tewari and Skoropad (1981) recorded conidium sizes of Canadian isolates to be 22.3 to 26 µm in length and 13.6 and 22.3 µm in width, with averages of 24.9 and 19.5 µm respectively. From these data, it can be assumed that the conidia of *P. arborescens* fall within the ranges of 11.4 to 29 µm in length and 8.5 to 22.3 µm in width. The conidia of *P. cristata* recorded on *Papaver* spp. are generally found to be larger than those of *P. arborescens*. A mean length of 21 µm, and width of 18.6 µm, was recorded by Gaumann (1923), from a specimen from *Pap. argemone*. In another study, isolates of *P. cristata* taken from *Pap. argemone* varied in mean length between 22.7 and 24.7 µm, and mean width between 18.4 and 20.8 µm (Gustavsson 1959). These data displays a high degree of overlap in conidium dimensions between the two species, which could be attributed to environmental and host genotypic differences.

The host range of the two oomycete species in question also exhibits a degree of overlap. *Peronospora arborescens* has been recorded as a pathogen of the plant species *Papaver rhoeas* L. (Berkeley 1846; Gustavsson 1959), *Pap. somniferum* (Behr 1956; Kothari & Prasad 1970; Yossifovitch 1929), *Pap. dubium* L. (Cotterill & Pascoe 1998; Cotton 1929; Gustavsson 1959), *Pap. argemone* L. (Cotton 1929), *Pap. nudicaule* L. (Alcock 1933; Cotterill & Pascoe 1998), *Pap. setigerum* L. (Behr 1956), *Pap. alpinum* L., *Pap. caucasicum* Bieb. (Francis 1981), *Argemone mexicana* L. (Maiti & Chattopadhyay 1986), and many species of *Meconopsis* (Alcock 1933; Cotton 1929; Reid 1969). *Peronospora cristata* has been recorded as infecting *Pap. hybridum* L. (Constantinescu 1991), *Pap. argemone* (Gaumann 1923; Gustavsson 1959), and *M. cambrica* Vig (Reid 1969). It should be noted that Kothari and Prasad (1970) tested *Pap. rhoeas* and *A. mexicana* and were unable to

infect using isolates of *P. arborescens*, suggesting the host range of species may vary with pathotype.

These comparisons indicate that clarification of the exact taxonomic status of *P. arborescens* and *P. cristata* is required. Use of molecular techniques in conjunction with biological observations, as advocated by Hall (1996) may provide some of the answers to this question. As *P. cristata* has not previously been recorded on *Pap. somniferum*, all further references to downy mildew on *Pap. somniferum* in this review of literature will refer to *P. arborescens*.

2.2.3. Disease cycle

Peronospora arborescens is thought to propagate itself via two methods, sexual reproduction of the oomycete to produce oospores, and asexual reproduction to form conidia (Behr 1956; Francis 1981; Kothari & Prasad 1970; Yossifovitch 1929). The method(s) of survival of the oomycete in the absence of the host plant and the subsequent primary infection of new hosts in the following season(s) are thought to involve the oospores of the oomycete and/or vegetative survival as mycelia. The means by which the oomycete survives in Tasmania have yet to be determined.

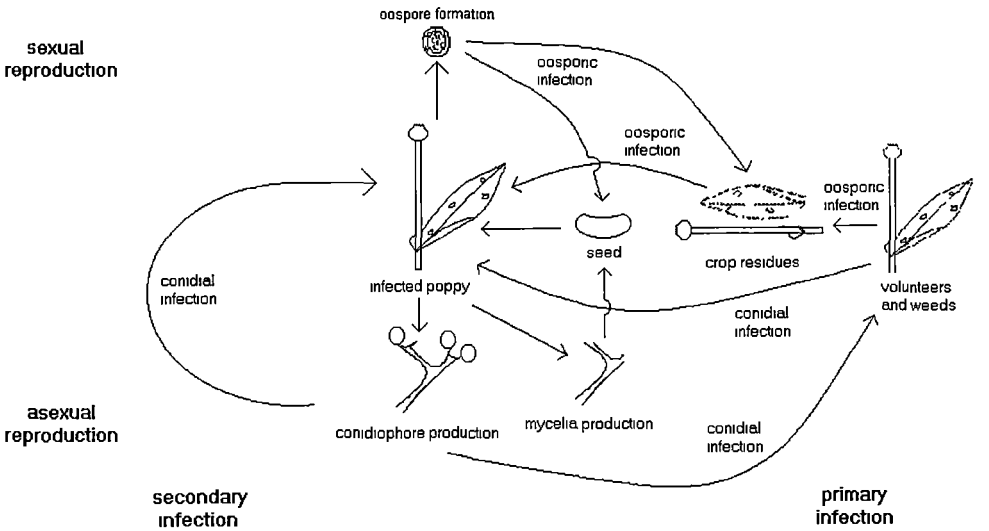


Fig. 2.7 Possible disease cycle for downy mildew of *Papaver somniferum* L. caused by *Peronospora arborescens* (Berk.) Casp.; constructed from previous research (Alavi 1975; Behr 1956; Kothari & Prasad 1970; Yossifovitch 1929).

Asexual reproduction

Asexual reproduction by *P. arborescens* takes place through the production of conidia (Fig. 2.7), which are important for secondary spread of the oomycete during the growing season (Behr 1956). Secondary spread allows the rapid development of epidemics in the presence of a susceptible host species, and suitable environmental conditions.

Conidia are formed on conidiophores, which protrude through the stomata of the host plant on the abaxial leaf surfaces (Behr 1956; Yossifovitch 1929). These conidiophores are external to the host plant, and thus allow rapid dissemination of conidia into the environment. The dissemination of conidia can take place via rain-splash, wind (Kothari & Prasad 1970), or leaf vibration (Leach *et al.* 1982).

Following dissemination, conidia are deposited on the external surface of host plants, germinate and produce germ tubes (Behr 1956; Maiti & Chattopadhyay 1986; Yossifovitch 1929). When a germ tube comes into contact with the solid surface of the poppy leaf, the tip flattens and forms an appressorium to facilitate infection (Behr 1956; Maiti & Chattopadhyay 1986; Yossifovitch 1929). Penetration of the host is not observed to take place via the host's stomata (Behr 1956), but rather through the epidermis of the host plant by the formation of infection or penetration pegs (Behr 1956; Maiti & Chattopadhyay 1986; Yossifovitch 1929). Evidence taken from other species within the *Peronospora* suggest that infection tends to take place over epidermal junctions, possibly because these are the points of minimum physical resistance (Chou 1970; Mence & Pegg 1971). Once invasion of the host has taken place, the mycelia of the oomycete grows intercellularly and feeds on host cells via the production of haustoria (Behr 1956; Maiti & Chattopadhyay 1986; Yossifovitch 1929).

The conidia of *Peronospora* spp. are relatively short lived. Conidia reportedly survive periods of days to months, with the actual time period highly dependent upon climatic conditions. The conidia of *P. arborescens* retained viability for 17 days when stored at 5 to 7 °C (Kothari & Prasad 1970). Longer periods of survival have been recorded for other *Peronospora* species. For example, the conidia of *P. viciae* remaining on sporulating lesions on the host plant reportedly survived for up to 22 days (Pegg & Mence 1970). When buried in the soil, the conidia of *P. tabacina* and *P. parasitica* were found to survive for several months (Kröber 1969).

The rate of secondary spread within poppy crops, due to conidia, is a major factor in determining the level of yield loss. Surveys in India found the incidence of

secondary infections to vary from 25 to 75 % of crop plants (Thakore *et al.* 1980), resulting in average yield losses of 19.2 % for latex and 12.6 % for seed (Thakore *et al.* 1980). Further work observed yield losses between 17 and 22.8 % for latex and 12.9 to 14.8 % for seed, due to secondary infection (Thakore *et al.* 1983). The significance of secondary spread on epidemics is highlighted by results obtained for the related pathogen, *P. destructor* Berk. (Casp.) ex Berk., the causal agent of onion (*Allium cepa* L.) downy mildew. Under favourable climatic conditions, *P. destructor* is capable of reaching 100 % disease incidence due to secondary spread within three generations (Jesperson & Sutton 1987). Each generation is observed to take as little as 10 days (Hildebrand & Sutton 1982). Therefore from initially low inoculum levels, a crop can become totally infected with *P. destructor* in a little as 30 days. Rapidly developing epidemics have also been recorded for *P. schachtii* Fuckel on beet crops (*Beta vulgaris* L.; Leach 1931).

These results show that the principal role of conidia is to facilitate the rapid secondary spread of downy mildew under favourable environmental and host conditions. They also show that conidia are unlikely to be utilised as means of long-term survival by *Peronospora* spp. due to their short survival period.

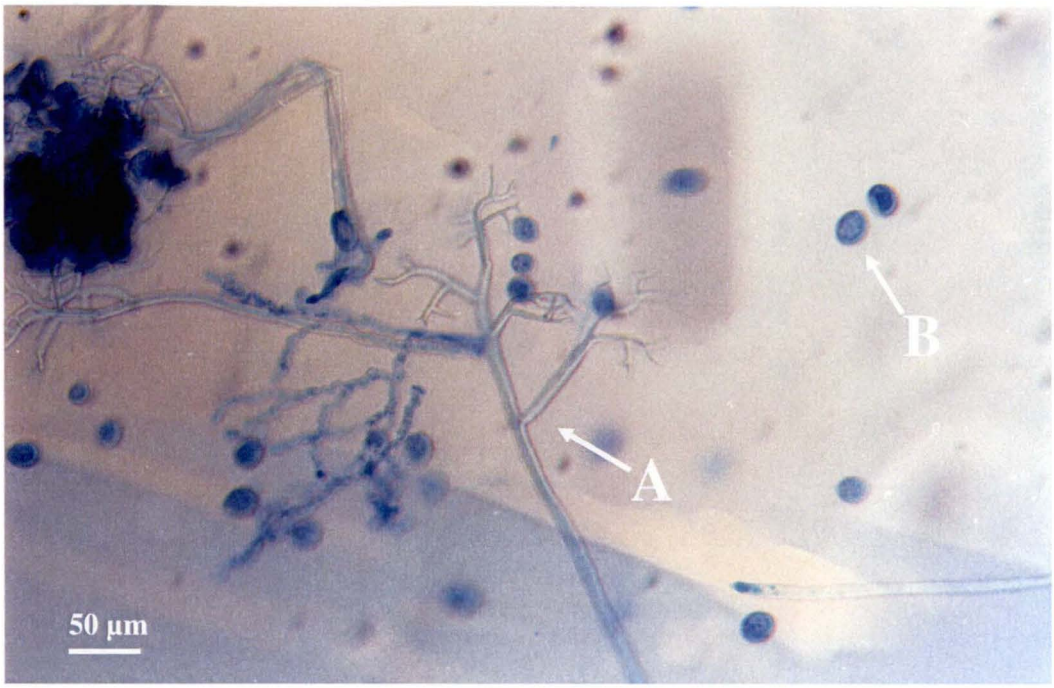


Fig. 2.8 Conidiophore and conidia of the causal organism of downy mildew of oilseed poppy. A) conidiophore; B) conidia. Stained with aniline blue (0.05 %).

Fig. 2.9 Conidia of the causal organism of downy mildew of oilseed poppy. Stained with aniline blue (0.05 %).

Sexual reproduction

The sexual reproduction of *P. arborescens*, like all oomycetes, takes place through the production of oospores (Fig. 2.7). The production of oospores by *P. arborescens* may enable the creation of genetic diversity within fungal populations, which does not occur under asexual reproduction. The other major role of oospores may be as resting spores capable of surviving long periods in the absence of the host plant and subsequently providing a source of primary inoculum in the following growing season (Behr 1956; Yossifovitch 1929). This is the case in India where studies of epidemics have used sites infested with oospores as sources of primary inoculum (Doshi & Thakore 2002).

Oospores are formed by the fertilisation of the female-type sexual organ of the oomycete (oogonium) by the male-type organ (antheridium) within the host plant (Behr 1956). Following fertilisation, the oospore forms within the oogonium, which eventually disintegrates around the oospore (Behr 1956). The major site of oospore formation within the host plant has been observed to be just below the epidermis of the foliage (Behr 1956). It has been suggested that some oospore formation may also take place within the seed capsule of the host plant (Alcock 1933). The oospores remain within the crop residues and tend to become buried in the soil during the decomposition of the host tissue. Oospores are thought to infect new hosts via the formation of germ tubes that penetrate the host tissue, as is the case with other members of this genus (Morgan 1978; Shaw 1981; Tommerup 1981; van der Gaag & Frinking 1996b). This has yet to be observed for *P. arborescens*.

The sexual nature of *P. arborescens* is thought to be homothallic, and thus consists of only one mating type that reproduces through intra-type crosses (Behr 1956). This is the only recorded report detailing the sexual nature of this oomycete. However, many other species within the *Peronospora* are both homothallic and heterothallic (i.e., require crosses between different mating types for sexual reproduction). Both homothallism and heterothallism have been demonstrated in *P. parasitica* (de Bruyn 1937; Kluczewski & Lucas 1983; McMeekin 1960; Sherriff & Lucas 1989). For heterothallic isolates two distinct mating types have been found, and the absence of one type has been found to prevent the formation of oospores (Kluczewski & Lucas 1983; Moss *et al.* 1994; Sherriff & Lucas 1989). Heterothallism has also been demonstrated in *Peronospora effusa* (Grev. Ex Desm.) Ces., the causal agent of downy mildew of spinach (*Spinacia oleracea* L.). However, no evidence of homothallism has been found for *P. effusa* (Inaba & Morinaka 1984). The existence of heterothallic populations in other *Peronospora* species, coupled with the possibility of distinct races occurring within the species *P. arborescens* (Alcock 1933; Kothari & Prasad 1970), indicates that distinct heterothallic isolates of *P. arborescens* might occur and may be present in Tasmania. This would greatly decrease the chances of oospore formation, unless both mating strains of the oomycete were present within the same geographic regions in roughly equal proportions (Inaba & Morinaka 1984; Michelmore 1981). An absence of one mating type within a heterothallic population would make oospore production impossible.

A few attempts have been made to induce germination of the oospores of *P. arborescens*, with limited success. Behr (1956) tried many different techniques, including exposure to heat, poppy juice solutions, natural weathering and acid degradation, without success. Kothari and Prasad (1970) attempted a similar set of

techniques utilising stimulatory and/or weathering conditions involving immersion in water, chemicals, poppy leaf extract, poppy soil leachate, soil extract, cow dung infusions and extracts of wet soil or root exudates. Weathering to induce germination of oospores was trialed using alternating heating and chilling, alternating wetting and drying, or varying temperatures. None of the above were found to induce oospore germination (Kothari & Prasad 1970). Inducing germination of oospores has proved difficult for the majority of the members of the genus *Peronospora* (McKay 1957; McMeekin 1960). However, germination has been observed for some species in the genus *Peronospora*. The oospores of *P. destructor* were induced to germinate through treatment with a potassium permanganate solution (McKay 1937; McKay 1957). The role of the potassium permanganate was believed to be in weathering the oospore wall, and thus increasing its permeability to water. *In situ* germination of oospores has also been observed for *P. destructor,* with oospores highly weathered at the time of germination (Berry & Davis 1957). Pathak *et al.* (1978) were able to induce the germination of the oospores of *P. manshurica* by washing with running distilled water, followed by shaking in distilled water. The oospores of *P. parasitica* have been induced to germinate on an agar mixture of host root exudate and soil extract (Jang & Safeeulla 1990b). Oospore germination has also been observed for *P. viciae* (van der Gaag & Frinking 1996a).

Once morphologically mature in appearance, a period of latency or physiological maturity occurs prior to oospores becoming capable of infecting host plants. This has been observed for *P. arborescens* (Kothari & Prasad 1970), as well as other *Peronospora* species (McKay 1957; Tommerup 1981; van der Gaag & Frinking 1997a; van der Gaag & Frinking 1997b). Sowing disease free seed in plots contaminated by *P. arborescens* oospores, was found to result in no crop infection in

the first year (Kothari & Prasad 1970). However, re-sowing of the plots in the second season following contamination was found to produce systemically infected seedlings from the age of 21 days after sowing onwards (Kothari & Prasad 1970). A more detailed study was undertaken on the oospores of *P. viciae* by van der Gaag and Frinking (1997c). The level of germinability increased from zero to an optimum at a time period that was determined by the conditions of incubation. The highest level of observed germinability was found with oospores incubated at 5 °C and a relative humidity of 30 % after 300 days incubation (van der Gaag & Frinking 1997a). In a later study, moist conditions were shown to reduce the period of oospore dormancy (van der Gaag & Frinking 1997b). McKay (1957) also indicated that a period of maturity was required by the oospores of *P. destructor* following their detachment from the host tissues with oospores reaching full maturity after four years. Contrary to the above evidence, the oospores of *P. arborescens* have been observed to induce infection in the same season as formation (Rathore *et al.* 1987). However, no reference to the exact time of storage or the extent of weathering of the oospores was made.

Arguments exist both for and against sexual and asexual production being antagonistic processes in the genus *Peronospora.* Murphy and McKay (1926) observed that conidia and oospore production were antagonistic processes for *P. destructor.* It was theorised that this was due to the higher nutritional requirements of oospore production. It has also been observed that when conditions are unfavourable for conidium production for *P. viciae*, oospore production begins (Pegg & Mence 1970). Tommerup (1981) noted that reductions in the level of conidiophore production occur when oospore production takes place in downy mildews. Populer (1981) theorised that oospores are formed when conditions are

unfavourable for conidium production. Conversely, no antagonism was found between the two processes in *P. parasitica* (McMeekin 1960). Oospores have been observed to form on the leaf spots of infected leaves at the same time as conidia for *P. arborescens* (Kothari & Prasad 1970; Scharif 1970).

The length of time that the oospores of *P. arborescens* are able to survive under natural conditions has not yet been determined. Kothari and Prasad (1970) observed that sites contaminated with oospores were still infective three years after initial contamination. Alavi (1975) recommended crop rotations of at least three years, and preferably four to five years, to prevent oosporic infection of seedlings. For other *Peronospora* species much longer oospore survival periods have been recorded. The oospores of *P. viciae* have been found to be capable of surviving in the soil for up to 15 years (Olofsson 1966). Periods of up to 25 years have been recorded for the oospores of *P. destructor* (McKay 1957).

These results indicate the role of oospores in the spread of the downy mildew pathogens. The ability to survive long periods in the absence of living host tissue suggests that oospores contribute to the long-term survival of *Peronospora* spp. Likewise, the slow rate of maturation of oospores signifies that they are unlikely to be involved in the rapid secondary spread of *Peronospora* spp.

Methods of overwintering

In Tasmania the means by which *P. arborescens* survives the winter period in the absence of cultivation of opium poppies is unknown. Cotterill and Pascoe (1998) suggested that volunteer ('regrowth') plants and/or alternative *Papaver* spp. growing

in the winter months may act as reservoirs for the parasite during this period. These would then serve as the sources of primary inoculum for the developing crop in the new season. The other main suggested methods for the survival of *P. arborescens* during the absence of the host plant are as seed borne inoculum, or on the residues of previous crops. It should be noted that downy mildews usually have more than one mode of overwintering and the predominant method may vary from region to region (Populer 1981).

Seed-borne inoculum

Seed-borne inoculum is advantageous to obligate parasites as it ensures constant association between host and parasite (Safeeulla & Shaw 1964). The status of seed-borne infection for *P. arborescens* is questionable at present, with arguments both for and against its existence.

Yossifovitch (1929) stated that there was no evidence of seed-borne infection. In support of this Kothari and Prasad (1970) argued that primary infection due to seed-borne inoculum does not exist. When seed collected from highly symptomatic plants was sown in pot and field trials, no infection was observed on 50,000 plants (Kothari & Prasad 1970). In addition, seeds did not form in poppy capsules systemically infected by the oomycete. It was therefore concluded that seed-borne infection does not exist for poppy downy mildew, as infection of the floral parts of the plant induced seed abortion (Kothari & Prasad 1970). Maiti and Chattopadhyay (1986) also reported that seed-borne infection does not exist in opium poppies, although they provided no evidence to support this assertion.

Conversely, Alcock (1933), Behr (1956) and Alavi (1975) provided evidence for seed-borne infection. The mycelia of *P. arborescens* have been observed to infect the seed capsules of *Meconopsis* spp. (Alcock 1933). This mycelial infection has also been observed to result in the formation of oospores within the seed capsule, surrounding the seeds themselves and sometimes attaching to the seed coat. The author therefore reasoned that seed-borne infection does take place in *Meconopsis* spp. Preliminary studies undertaken in Germany were also highly indicative of seed-borne inoculum (Behr 1956). Microscopic examination revealed the presence of mycelia within the ovules of poppy plants, which was reasoned to constitute evidence of seed infection (Behr 1956). It was argued that the inoculum took the form of the mycelia itself inside the seed rather that oospores, as oospores were assumed to form in dying tissue rather than living seed tissues. Alavi (1975) collected and sowed seed from infected crops in areas isolated from other poppy crops, and with no previous history of poppy. The resultant crops were severely infected by *P. arborescens*. However, no evidence was presented to suggest the form in which this inoculum occurred.

Seed-borne infection has been recorded for a number of members of the genus *Peronospora*. Soybean seeds are commonly found encrusted with the oospores and mycelia of *P. manshurica* on the surface and below the seed coat (Jones & Torrie 1946; Pathak *et al.* 1978; Roongruangsree *et al.* 1988), leading to seedling infection (Jones & Torrie 1946). Embryonic infection of brassica seeds by *P. parasitica* mycelia has been observed (Achar 1995; Jang & Safeeulla 1990b), with subsequent seedling infection (Achar 1995). *Peronospora parasitica* has also been observed to form oospores on the surface of seeds with subsequent seedling infection (Vishunavat & Kolte 1993). Oospore infection of seeds has also been observed for

P. viciae, in pea (Mence & Pegg 1971), and *P. ducometi* Siem. and Jank., in *Fagopyrum esculentum* Moench (Zimmer *et al.* 1992). Infection by mycelium of *P. destructor* of the floral parts of onion has been recorded and used as an indicator of seed-borne infection (Cook 1930). The presence of seed-borne inoculum for these related species strengthens the argument that *P. arborescens* is able to survive in this manner.

Crop residues

It is generally agreed that *P. arborescens* is able to survive in the absence of host plants on the residues of previous hosts. The incidence of *P. arborescens* infection within a crop is often reported to be at its highest when successive crops are sown in the same growing areas (Kothari & Prasad 1970; Yossifovitch 1929). Yossifovitch (1929) found that when fragments of diseased capsules from poppy crops were incorporated into the soil prior to the sowing of a fresh crop, high incidences of downy mildew resulted. No investigation was made into the form of inoculum present in this study. Cotton (1929) agreed with these findings, noting that the oomycete could also survive on the leaf material from previous crops. It was noted that the oospores of *P. arborescens* were able to remain viable in rotting plant residues and therefore might make good sources of primary inoculum in the following seasons (Alcock 1933).

Despite an inability to induce oospore germination by artificial means, Behr (1956) was able to induce infection of new seedlings by incorporating oospores into soil mixtures. This oospore mixture was also observed to contain downy mildew mycelia. Behr (1956) argued that both the oospores and the mycelia, in a dormant

phase, were able to survive on the residues of crops and then infect new crops in subsequent years. Kothari and Prasad (1970) and Rathore *et al.* (1987) also showed that infection of plants grown from disease free seed could be induced by sowing into soil containing plant material infested with oospores of *P. arborescens.*

Regrowth and alternative *Papaver* spp.

Although recognised as a means of overwintering, little research has been published on the significance of survival mechanism provided by regrowth oilseed poppies and alternative hosts to the disease cycle of poppy downy mildew. This is also the case for downy mildew species in general, with most references to this form of survival being vague (Renfro & Bhat 1981). Cotterill and Pascoe (1998) reasoned that the presence of alternative hosts for *P. arborescens*, such as *Papaver* spp. weeds of opium poppy crops, could constitute a means of overwintering for the oomycete. The growth of these weeds over the winter months would provide a living host on which the oomycete could survive. This would especially be the case in the absence of oospore production (Cotterill & Pascoe 1998).

2.2.4. Climatic effects

Many different weather variables are known to affect the spread and infection of opium poppy plants by *P. arborescens*, including ambient temperature, relative humidity, free moisture, rainfall, wind and light conditions. Knowledge of the manner in which environmental conditions affect the various stages of the disease cycle of a pathogen can be used to enhance the control of disease outbreaks. In some situations the microclimate of crops can be modified (e.g. choice of planting site,

manipulation of irrigation, altering plant density) to prevent the occurrence of favourable conditions for disease spread.

Alternatively the above knowledge can be used to construct disease forecasting models. These models can use measured and/or forecasted weather conditions to predict the extent and/or timing of disease spread. This information can then be used to strategically apply chemical fungicides for maximum control and minimal usage (Jesperson & Sutton 1987; Scherm & van Bruggen 1993). Such a system might have advantages over the current calendar-based system employed in the Tasmanian poppy industry, which leads to fungicide application at set times independent of requirement. Forecasting models have been constructed for many different downy mildew species, based around several different aspects of their individual disease cycles.

Temperature

Field studies have correlated non-systemic infections, caused by conidia, with minimum temperatures in the range of 5 to 11 °C and maximum temperatures in the range of 20 to 26 °C (Doshi & Thakore 2002). There is no published information on the direct effect of temperature on the process of asexual sporulation for *P. arborescens*. However, studies of the effects of temperature on sporulation have been undertaken for the oomycete *P. destructor*. This information was used to create and modify the forecasting model DOWNCAST. Sporulation of *P. destructor* has been observed to take place at temperatures in the range of 4 to 24 or 28 °C (Hildebrand & Sutton 1982; Hildebrand & Sutton 1984b; Yarwood 1943), and the optimum temperature for sporulation is believed to be 13 to 14 °C (Hildebrand &

Sutton 1984b; Yarwood 1943). In addition to the temperatures at the time of formation, the temperatures of the preceding day also affect sporulation. For *P. destructor*, mean temperatures above 24 °C the previous day were found to inhibit sporulation (Hildebrand & Sutton 1982). Periods of high temperature the previous day are also important. Temperatures of 27, 28, 29 and 30 °C, or greater, for in excess of 8, 6, 4 and 2 hours respectively were inhibitory to sporulation the following day (de Visser 1998). This effect of temperature on sporulation was also observed in *P. viciae*, with temperatures outside the range 4 to 20 °C during sporulation and/or 20 to 24 °C prior to sporulation being inhibitory (Pegg & Mence 1970). This interaction between temperature and sporulation may also occur for *P. arborescens.*

Survival of conidia has been observed to be increased by storage at low ambient temperatures (Kothari & Prasad 1970). Conidia remained viable for up to 17 days under laboratory conditions when stored at 5 to 7 °C, but viability was observed to be rapidly lost when conidia were stored at higher temperatures. Behr (1956) observed conidia to remain viable under laboratory conditions for up to 12 days when stored at 12 °C. A similar pattern has also been observed for *P. tabacina, P. farinosa* (Fr.) Fr., *P. parasitica* (Kröber 1969), and *P. viciae* (Pegg & Mence 1970).

Early records of conidium germination indicated that the conidia of *P. arborescens* were able to germinate within the range of 4 to 26 °C (Yossifovitch 1929), with optimum germination occurring in the range 17 to 18 °C. Behr (1956) observed that conidia were able to germinate within the temperature range 15 to 23 °C with an optimum temperature of 19 °C. Behr (1956) also observed some late germination from conidia exposed to 2 °C, however, these conidia were observed to be of varying diameters and misshapen. Kothari and Prassad (1970) observed germination in the

range 5 to 20 °C, with optimum levels occurring in the range 15 to 20 °C. Doshi and Thakore (1993) found general agreement with previous results, stating that the optimum temperature for conidium germination was in the range 14 to 20 °C. Therefore although the optimum temperatures are 15 to 20 °C, germination has been recorded within the range of 2 to 26 °C. The optimum temperature for host infection by conidia occurs within the range 12 to 17 °C (Behr 1956). This is slightly lower than the observed optimum for the germination of conidia, which suggests that host penetration and/or colonisation require lower temperatures. However, some authors doubt that there are any real differences between temperatures for germination and the other stages of infection (Populer 1981). It is possible that the observed difference is merely an artefact of experimental conditions.

No published information exists on the effects of temperature on sexual reproduction of *Peronospora* spp. infecting *Papaver spp.* Therefore inferences must be made from research undertaken on the oospores of the members of the genus *Peronospora.*

The formation of oospores is probably favoured by cool to moderate temperatures. *Peronospora viciae* oospore formation has been recorded in the temperature range 5 to 20 °C (van der Gaag & Frinking 1996b; van der Gaag *et al.* 1993). Some variation in the optimum temperature for oospore formation is observed in this species. Studies into *P. viciae* f.sp. *fabae* found optimum oospore formation at 10 to 15 °C (van der Gaag *et al.* 1993), while the optimum for *P. viciae* f.sp. *pisi* was 20 °C (van der Gaag & Frinking 1996b). For *P. trifoliorum*, the highest level of oospore production was observed at 16 °C, compared to 20 and 24 °C (Hodgden & Stuteville 1977).

There is little published work on the effect of temperature on survival of oospores. van der Gaag and Frinking (1997a) found no consistently significant difference in survival of the oospores in soil of *P. vicae* over the temperature range 5 to 20 °C. No other published information is available.

Cool temperatures generally favour the germination of oospores of *Peronospora* spp. Germination of oospores of *P. destructor* have been recorded in the range 2 to 20 °C, with an optimum of 20 °C (McKay 1957). For *P. viciae*, germination occurred between 5 and 20 °C, with optimum levels between 5 and 10 °C (van der Gaag & Frinking 1997a). Similarly, conidium germination of *P. viciae* occurred in the range 1 to 24 °C with an optimum of 4 to 8 °C (Pegg & Mence 1970). The similarity between the temperature profiles of oospore and conidia germination is also apparent in *P. parasitica.* Oospores of *P. parasitica* germinate between 13 and 30 °C, with an optimum of 23 °C (Jang & Safeeulla 1990a). For conidia, germination occurs in the range 5 to 30 °C, with an optimum of 20 °C (Achar 1998; Mehta *et al.* 1995; Singh 1997).

It should be noted that the role of temperature might be secondary to other environmental factors, modifying their effect rather than having a direct effect itself (de Weille 1963).

Relative humidity

It has long been known that relative humidity plays a vital role in the formation of conidia of *P. arborescens.* Yossifovitch (1928) observed that under field conditions, high humidity was required to induce sporulation. Doshi and Thakore (2002)

concluded that secondary infections were favoured by mean relative humidities in the range 70 to 93 %. Kothari and Prasad (1970) found that high relative humidities were able to induce sporulation in detached leaves within 12 to 24 hours. No detailed studies have been undertaken to determine the threshold values of relative humidity for sporulation of *P. arborescens*. However, for other *Peronospora* species the greatest level of sporulation occurs at 100 % relative humidity. Threshold values for sporulation vary among species, from 90 % reported for *P. destructor* (Develash & Sugha 1996; Yarwood 1943), *P. viciae* (Olofsson 1966), and *P. tabacina* (Hill 1961), to 97 % for *P. trifoliorum* (Fried & Stuteville 1977) and 98 % for *P. parasitica* (Paul *et al.* 1998). In the genus *Peronospora*, the most detailed studies of the effect of relative humidity on sporulation have been undertaken for *P. destructor*. Hildebrand and Sutton (1982) observed a higher threshold value for sporulation (95 %) than reported by Yarwood (1943). To induce sporulation it was found that humidities greater than the threshold level were required for a continuous period of at least four hours (Hildebrand & Sutton 1982). Hildebrand and Sutton (1982) also found that relative humidity and temperature interact to influence sporulation. The optimum temperature for sporulation was 14 °C at eight hours continuous humidity above 95 %, but 18 °C at the minimum of four hours of humidity (Hildebrand & Sutton 1984b). By analogy, the likely threshold value for the induction of sporulation of *P. arborescens* is 90 % relative humidity, or greater and that periods of continuous humidity greater than the threshold level must approach, or exceed four hours for sporulation to take place.

No research has been published as to the role of relative humidity on the dissemination of the conidia of *P. arborescens*. Studies with *P. tabacina* have shown that changes in relative humidity induce the release of conidia, rather than specific

levels of relative humidity (Hill 1960; Hill 1961; Pinckard 1942). This response has also been shown for the species *P. parasitica*, *P. geranii* Feck., and *P. halsteadii* Farl. (Pinckard 1942), *P. trifoliorum* (Fried & Stuteville 1977), and *P. destructor* (Leach 1982). Two theories exist for the explanation of this phenomenon. Pinckard (1942) described a twisting motion exhibited by the conidiophores under changes in atmospheric humidity, which resulted in the release of conidia into the atmosphere. This process has also been observed with *P. tabacina* (Hill 1961), *P. trifoliorum* (Fried & Stuteville 1977) and *P. viciae* (Falloon & Sutherland 1996). Hill (1961) found humidity changes in both directions were able to induce the release of conidia. The second model for conidium release revolves around the creation of electrostatic charges between conidia and conidiophores under saturated conditions (Leach 1976; Leach 1980; Leach 1982). Following changes in atmospheric humidity, these charges become static, weakening the bonds between the conidia and conidiophores and allowing violent release of the conidia. Under both of these models, conidium release typically exhibits a bimodal release pattern due to the decrease of relative humidity during the morning, and the increase during the onset of nightfall (Hill 1961). The fact that spore release is only detected during the morning was attributed to the fact that sporulation only takes place during the night (Hill 1961; Leach 1975). Therefore, the vast majority of spores formed would be released during the morning period, leaving few for release with the rising humidities of nightfall.

The effect of relative humidity on the survival of the conidia of *P. arborescens* is another area in which knowledge is currently lacking. Studies with other *Peronospora* species have shown that relative humidity has a mild effect on conidium survival following release. For the species *P. parasitica*, *P. farinosa* and *P. tabacina*, dry atmospheric conditions are more favourable for survival than moist

conditions (Kröber 1969). A more detailed study of *P. tabacina* and *P. destructor* has shown that the optimum relative humidity for conidium survival is approximately 53 % (Bashi & Aylor 1983). However, it is also noted that light plays a much more significant role in conidium survival than does relative humidity (Bashi & Aylor 1983).

Relative humidity may also play a role in the infection process of conidia. Yossifovitch (1929) observed that while free water was required for infection under laboratory conditions, high atmospheric humidity was sufficient for infection in the field. Behr (1956) showed that high levels of humidity were required for conidium germination to take place. Later research showed that conidium germination is greatest (100 % conidium germination) at 100 % relative humidity (Doshi & Thakore 1993). At 80 % relative humidity only 68.5 % of conidia germinated (Doshi & Thakore 1993). This is consistent with results obtained for other *Peronospora* species. Maximum levels of germination were obtained for *P. parasitica* (Achar 1998) and *P. destructor* (Develash & Sugha 1996) at 100 % relative humidity. Conversely, conidia of *P. trifoliorum* were found to be unable to germinate in the absence of free moisture, even at relative humidities of 100 % (Patel 1926). However, it should be noted that this study was undertaken under laboratory conditions.

No research has been published on the effect of relative humidity on oospores of *P. arborescens*. However, the effect of relative humidity on oospore formation has been studied for *P. viciae*. Relative humidities between 60 and 80 % were the most favourable for oospore formation (van der Gaag & Frinking 1996b). To the

knowledge of this author, no studies have been conducted into the effects of relative humidity on oospore survival or germination for the genus *Peronospora.*

Free moisture

Free moisture on leaf surfaces has an important influence on the formation of, and infection by, conidia of *P. arborescens* and the downy mildews in general. Apart from one study conducted on the oospores of *P. viciae*, very little research has been undertaken into free moisture and its effect on the sexual stages of the downy mildew disease cycle. In that study, oospores kept in moist conditions were shown to germinate more rapidly than those in dry conditions (van der Gaag & Frinking 1997a).

The effect of free moisture during sporulation has not been investigated for *P. arborescens.* However, for *P. trifoliorum*, the presence of free moisture on the leaves of host plants inhibited sporulation (Fried & Stuteville 1977). For *P. parasitica*, Hartmann *et al.* (1983) reported that while sporulation was not completely inhibited by the presence of free moisture, conidiophores were always observed to be adjacent to water droplets. Likewise, *P. destructor* has been found to not form conidiophores in areas covered with water films (Hildebrand & Sutton 1982; Sutton & Hildebrand 1985). Sutton and Hildebrand (1985) suggested that this inhibition of sporulation by free moisture may be due to interference to gas or metabolite exchange between the pathogen and the environment. Another possibility is that the presence of free moisture inhibits the apical growth of conidiophores (Sutton & Hildebrand 1985). The effect of free moisture on sporulation of

P. arborescens has not been published; however, it is possible that the inhibition described above also exists for *P. arborescens.*

Free moisture plays a prominent role in germination and/or infection involving conidia. However, the exact role of free moisture is contentious. Yossifovitch (1929) stated that free moisture was required for germination on glass slides, but in the field only moderate atmospheric humidity without free moisture accumulation was required. Conversely, Kothari and Prasad (1970) found that free moisture for a period of at least four hours was required for conidium germination and subsequent infection. The confusion surrounding the requirement of free moisture for infection may be based on the alternation of the terms infection and germination. Germination is only one part of the infection process, and does not require free moisture in order to take place (Doshi & Thakore 1993). However following germination, free moisture on the host's leaf surface is required for germ tube elongation and subsequent host infection. Germ tubes produced in the absence of free moisture were very short, and it was concluded that these would be unable to subsequently infect the host plant (Doshi & Thakore 1993). Therefore free moisture may actually be required for infection processes immediately following germination.

The role of free moisture in conidium infection has been investigated in more depth with other *Peronospora* species. Free moisture has been found to be a requirement for conidium infection for the species *P. tabacina* (Cruickshank 1961; Hill 1969), *P. destructor* (Develash & Sugha 1996; Hildebrand & Sutton 1982; Hildebrand & Sutton 1984c), *P. parasitica* (Hartmann *et al.* 1983; Mehta *et al.* 1995; Yarwood 1943), *P. viciae* (Olofsson 1966; Pegg & Mence 1970), and *P. trifoliorum* (Patel 1926). The period and rate of condensation on leaf surfaces have been found to be as

important for infection as the existence of leaf wetness. For *P. viciae*, Olofsson (1966) and Pegg and Mence (1970) reported that three and four hours of leaf wetness, respectively, were required for infection. For *P. destructor*, increasing the duration of leaf wetness above two hours has been found to increase the percentage of plants infected (Hildebrand & Sutton 1984c). For *P. destructor*, infection requires rapid rates of condensation on leaf surfaces (Hildebrand & Sutton 1984a). Slow rates of condensation have been associated with the death of conidia (Hildebrand & Sutton 1984a). Slow rates of condensation were suggestive of alternating wetting and drying of the leaf surface, and thus the conidia (Hildebrand & Sutton 1984a). Conversely, rapid rates of condensation were associated with continuous wetting of leaf surfaces (Hildebrand & Sutton 1984a). To enable conidium survival without infection, conidia must be exposed to continual dry periods (Hildebrand & Sutton 1984a).

The requirement of free moisture may be, at least in part, due to the presence of auto-inhibitors within downy mildew conidia. It has been noted that germination begins with the hydration of dried conidia, which has been synchronised with the removal of water-soluble inhibitors (Tommerup 1981). Washing conidia of *P. destructor* has been found to increase the level of germination that occurs, which may be due to removal of auto-inhibitors (Abd-Elrazik & Lorbeer 1980). These inhibitors have been identified in the conidia of *P. tabacina* and are thought to inhibit the germination of immature conidia still attached to conidiophores (Shepherd & Mandryk 1962). The presence of auto-inhibitors in *P. arborescens* would nullify the argument that free moisture is only required for infection by, and not for the germination of, conidia for that species.

Rainfall

Other than vague references of cool rainy conditions favouring conidium infection (Yossifovitch 1929), no reference to the effect of rainfall on the disease cycle of *P. arborescens* has been published. Rainfall affects both the asexual and sexual reproduction of other downy mildews. Rainfall is a major influence on the asexual processes of sporulation and dissemination during asexual development. Rainfall may also play a role in the conidium infection process, and is also important for the sexual development of downy mildews.

Based on studies of onion downy mildew, rainfall appears to inhibit the sporulation process. Rainfall during sporulation causes damage to the developing conidiophores and/or conidia (Hildebrand & Sutton 1982; Yarwood 1943). It is probable that this damage is due to the physical forces exerted by rainfall. It may also result from the presence of raindrops on leaf surfaces that act to inhibit sporulation in the same manner as free moisture.

Rainsplash is believed to be an important mechanism for the dissemination of mature conidia. Observations of *P. tabacina* have shown that the physical vibration exerted by the impact of raindrops on sporulating leaves increased the amount of conidium release in any given period (Hill 1961). A similar effect has also been observed for *P. parasitica* (Hirst & Stedman 1963). Rainsplash was also important for the dispersal of the conidia of *P. viciae* (Stegmark 1994).

Rainfall may also affect asexual reproduction during the infection process due to the deposition of free moisture on leaf surfaces. Populer (1981) proposed that rainfall may actually be more important for occurrence of leaf wetness than dew deposition.

However, this is not a view shared by the majority of authors (de Visser 1998; Hildebrand & Sutton 1982).

Rainfall affects both the formation and maturation processes of downy mildew oospores. Periods of extended rainfall are thought to promote the formation of oospores by downy mildew species, at the expense of conidium formation (McKay 1957; Populer 1981). Rainfall within specific ranges for a given period has also been found to promote the maturation of the oospores of *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni (Tran Manh Sung *et al.* 1990). However, rainfall below or in excess of this range, has been found to be detrimental to the rate of oospore maturation for this oomycete (Tran Manh Sung *et al.* 1990). No research in this area has been reported for any members of the genus *Peronospora.*

Light

Light irradiation also influences the asexual and sexual stages of the disease cycle of *P. arborescens.* Light radiation has been observed to affect many stages of the asexual reproduction of downy mildew fungi, especially sporulation, conidium dissemination and conidium survival. Little work has been undertaken into the effect of radiation on the development of the sexual stage of *Peronospora* species.

While no detailed research has been conducted into the effect of light on the life stages of *P. arborescens*, the effect of light on the process of sporulation is well documented for other *Peronospora* species. Sporulation appears to be an irreversible, darkness-induced process, with only one to two hours darkness required for induction (Cohen 1976; Cruickshank 1963). Studies with *P. tabacina* have

suggested that the process is inhibited by a diffusible host factor, because light irradiated on leaves of a host plant was able to inhibit sporulation on other leaves maintained under dark conditions (Cohen 1976). Prior to the induction of sporulation, a period of light is required to allow induction to take place (Hill 1961; Yarwood 1937). Yarwood (1937) hypothesised from studies of *P. destructor* that this phenomenon may be related to the build up of carbohydrates by the host under light conditions providing the energy required for sporulation. It was shown that sporulation on leaves maintained under dark conditions for both 12 and 24 hours could be promoted by a single period of 12 hours of light (Yarwood 1937). The amount of sporulation is dependent upon the ratio of light to dark periods, with increased ratios resulting in lower levels of sporulation (Cohen *et al.* 1977; Uozumi & Kröber 1967). For *P. tabacina*, the optimum conditions for sporulation have been reported as 13 hours of light following by five hours of dark (Uozumi & Kröber 1967).

Several authors have reported light to be inhibitory during sporulation of downy mildew, including *P. arborescens* (Cohen 1976; Cruickshank 1963; de Weille 1961; Fried & Stuteville 1977; Hildebrand & Sutton 1984b; Yarwood 1937; Yarwood 1943), especially blue light (Cruickshank 1963; Michelmore 1981). de Weille (1961) noted that continuous ultra-violet (UV) light illumination allowed the formation of conidiophores, but not conidia of *P. arborescens.* Conidium formation was observed to take place in subsequent dark periods. Studies with *P. trifoliorum* showed that a short period of light halted sporulation for two to three hours, after which sporulation resumed (Fried & Stuteville 1977). Similarly, intermittent periods of light during sporulation of *P. tabacina* were found to reduce sporulation (Cruickshank 1963). Light during the initial stages of sporulation by *P. destructor*

has been shown to cause conidium deformation (Hildebrand & Sutton 1984b; Yarwood 1943). Conidium deformation has also been reported for *P. tabacina* (Cohen 1976). Light irradiation later in the development of the conidia of *P. destructor* was found to result in a reduction in sporulation (Hildebrand & Sutton 1984b).

The inhibition of sporulation by light may be a temperature dependent process. At temperatures below 20 °C much higher levels of light irradiation are required for the inhibition of the sporulation of *P. tabacina* (Cohen 1976). Light inhibition does not occur for *P. tabacina* below 15 °C (Cohen *et al.* 1977). It has been hypothesised that the low temperature may cause a conformation shift and inactivate a host photoreceptor, preventing the production of a sporulation inhibitor by the host (Cohen 1976).

The dissemination of the conidia of *P. arborescens*, with respect to solar radiation has yet to be studied. However for many fungi, including *P. destructor*, red and infra-red radiation have been found to induce spore release (Leach 1975; Leach 1976; Leach 1982; Leach *et al.* 1982). Spore release has been related to the electrostatic theory of spore release. Red and infra-red radiation have been shown to induce electrical charge changes on leaf surfaces, which could induce the release of conidia (Leach *et al.* 1982). The effects of light on sporulation and dissemination, coupled with the effects of relative humidity on spore release, have combined to produce a characteristic diurnal release pattern for the conidia of downy mildew species. This pattern was first observed in *P. destructor* by Yarwood (1937). It has since been recorded for other species within the *Peronospora*, including *P. tabacina* (Hill 1961). The production of oospores at night, and the subsequent decreasing

relative humidities and increasing levels of solar radiation in the morning, results in peak spore releases in early to mid-morning under typical conditions.

The relationship between UV exposure and germination is a bell-shaped curve. Low levels of radiation have been observed to promote conidium germination (de Weille 1961). Germination increases with increasing UV dose to an optimum point (de Weille 1961). However, increases in UV dose beyond this point are found to decrease the germinability of conidia (de Weille 1961). Similar results have been obtained for the period of exposure to UV light. It has been found that UV light is able to enhance the germination of the conidia of *P. arborescens* for exposure periods of up to 30 min (Doshi & Thakore 1993). However beyond this period, UV light exposure becomes lethal to conidia and reduces the secondary spread of the oomycete (Doshi & Thakore 1993). In a study of *P. destructor* and *P. tabacina*, solar radiation was found to be more lethal to conidia than high ambient temperatures (Bashi & Aylor 1983). In the same study, the threshold level of radiation intensity for conidium survival was found to be low with respect to natural light conditions. In a study of *P. tabacina*, UV light was demonstrated to be the major fungicidal component of solar radiation (Rotem *et al.* 1985). Conservely, Kothari and Prasad (1970) did not observe significant changes in the level of germination of conidia of *P. arborescens* when exposed to natural daylight, compared to darkness (Kothari & Prasad 1970). However, no reference was made to the intensity of 'natural daylight' to enable comparison with other studies.

Formation of oospores by some *Peronospora spp.* is favoured by long photoperiods in the field. The highest level of oospore production for *P. trifoliorum* occurred

under continuous 6,500 lux light intensity (Hodgden & Stuteville 1977). Oospore production decreased under decreased photoperiods.

Oospore germination by *Peronospora spp.* may also be affected by light conditions, however, reports as to the effect of light are inconsistent. Periods of alternating light and dark were observed to favour the germination of oospores of *P. parasitica*, in comparison with continuous dark and continuous light conditions (Jang & Safeeulla 1990a). No information was provided by the authors pertaining to the length of each light and dark period. For *P. vicae*, 16 hours of light at an intensity of 10 $J.s^{-1}.m^{-2}$ was found to negatively affect oospore germination (van der Gaag & Frinking 1996b). From these results it can be assumed that continuous light periods are detrimental to the germination of oospores.

Wind

No studies have been reported on the effect of air movement on the disease cycle of *P. arborescens*. However for other *Peronospora* spp. and related oomycetes, wind is important for the dissemination of conidia.

Following the formation of conidia during sporulation, wind has been observed to induce the release of conidia into the atmosphere for dissemination (Populer 1981). Leaf vibrations caused by air movement have been found to increase the amount of conidium release for *P. destructor* induced by favourable moisture and light conditions (Leach *et al.* 1982). Studies on *P. tabacina* have shown that it is not the wind speed that induces conidia release but rather changes in the wind speed (Hill 1961). This has also been shown for other downy mildew species (Populer 1981).

Once conidia have been released from conidiophores, wind plays a pivotal role in their transport to new hosts. Studies of onion downy mildew have shown that wind borne conidia from outside a crop can act as the primary source of inoculum (Newhall 1938). This transport can be over short, medium and long distances from the initial source. A key example of long distance dispersal of oomycetes took place in Europe in the early 1960's where tobacco blue mould was observed to progress as much as 400 km.month^{-1} with wind currents (Populer 1981). Currently in the United States, wind current monitoring is used to predict the spread of tobacco blue mould each season (Main *et al.* 1998). It should be noted, however, that the strength of wind currents may also limit the distances conidia are able to travel, due to the infliction of physical damage (Stuteville 1981).

Disease models

Disease forecasting models have been developed for a number of different pathosystems involving oomycetes, including the downy mildews. Downy mildew pathosystems that have currently had forecasting models developed for them include downy mildew of grape (*Plasmopara viticola*; Blaise & Gessler 1990; Madden *et al.* 2000; Orlandini *et al.* 1993; Rosa *et al.* 1995), hop (*Pseudoperonospora humuli*; Johnson *et al.* 1994; Johnson *et al.* 1983; Royle 1973) and lettuce (*Bremia lactuca*; Kushalappa 2001; Scherm *et al.* 1995; Wu *et al.* 2001a; Wu *et al.* 2001c; Wu *et al.* 2002). Within the genus *Peronospora* only two pathosystems currently have forecasting models developed for them. These are downy mildew of onion, caused by *P. destructor* (Jesperson & Sutton 1987), and tobacco blue mould, caused by *P. tabacina* (Main *et al.* 1998).

Three disease models have been developed for the prediction of the asexual development of *Peronospora* spp. epidemics within fields, all for onion downy mildew, caused by *P. destructor*; 'DOWNCAST', 'ONIMIL' and 'ZWIPERO'. DOWNCAST, was initially developed for Canadian growing conditions (Jesperson & Sutton 1987), but has since been further adapted for the Netherlands (de Visser 1998), and has been tested in Australia (Fitz Gerald & O'Brien 1994; Mac Manus *et al.* 1996; Mac Manus & Harper 1999). DOWNCAST is divided into two sub-units dealing with sporulation and infection respectively (Fig. 2.10). Sporulation predictions are based on nighttime temperature, relative humidity and rainfall levels, and daytime temperatures. Nighttime temperatures must be between 4 and 24 °C, while relative humidity must be above 95 % for at least four hours, dependent upon the mean temperature. In addition, rainfall cannot be greater than 0.2 mm for the night period and daytime temperatures the preceding day, or days, cannot be high for extended periods (Fig. 2.10). Infection predictions are based on the length of leaf wetness periods with three to six hours of leaf wetness required (temperature dependent) in the morning following sporulation to allow infection. If morning infection does not occur a five-hour period of rapid dew deposition followed by three hours of leaf wetness is required for infection. A period of slow dew deposition is lethal to conidia, and if infection or conidium death does not occur prior, then conidia are assumed to decease after 72 hours. Overall predictions are a product of the semi-quantitative prediction of sporulation levels (based on the length of the relative humidity period and mean temperatures) and the binary prediction of infection.

The models ONIMIL and ZWIPERO are essentially quantitative adaptations of DOWNCAST. ONIMIL uses the qualitative structure of DOWNCAST and attempts to quantify the level of secondary infection occurring in the individual predicted periods of infection (Battilani *et al.* 1996a; Battilani *et al.* 1996b). If the conditions for sporulation are met then the relative level of conidia produced is calculated mathematically with temperature as an independent parameter and other parameters set based on the length of the high relative humidity period. Conditions for sporulation are identical to those of DOWNCAST (Jesperson & Sutton 1987), with the exception that the upper nighttime temperature limit for sporulation is 26 °C (Battilani *et al.* 1996b). Following sporulation, the survival rate of conidia produced is determined for subsequent 12 hour periods using a polynomial equation incorporating temperature and relative humidity as the independent variables, based on the work of Sutton and Hildebrand (1985). As with DOWNCAST, the survival rate of conidia is assumed to be zero if a period of slow dew deposition occurs. ONIMIL differs from DOWNCAST in that infection is predicted by ONIMIL in a given 12 hour period when a single continuous leaf wetness period is longer than the critical limit for the length of leaf wetness in that given period, which is calculated using temperature as the sole variable. If infection is predicted, the germination rate of conidia is determined using parameters based on the length of the leaf wetness period, and the temperature of infection. The overall level of infection predicted by ONIMIL is then the product of the germination rate and the number of surviving conidia at the time of infection.

The third model, the recently described ZWIPERO, also attempts to quantify the level of infection, but differs from DOWNCAST and ONIMIL in its use of relative scales for parameters rather than distinct limits (Friedrich *et al.* 2003). ZWIPERO

also differs in that it does not directly use relative humidity as a predictor of sporulation, but rather converts these values to vapour pressure deficit (VPD). Development rates for sporulation are calculated nightly using both temperature and VPD independently, based on defined mathematical functions. The development rate of VPD is multiplied by a factor of five when leaf wetness is present. If VPD rises above 1 hPa (i.e., relative humidity becomes too low) then the hourly calculated development rates are reset to zero. Sporulation is predicted to occur when the development rate calculated using temperature is greater than or equal to four, which is analogous to the four hours relative humidity required at optimum temperatures for sporulation under DOWNCAST. The relative number of conidia produced is then calculated as the product of the cumulative development rates from temperature and VPD. This number is then reduced by the proportion of injured conidia, which is proportional to the extent of nighttime rainfall. Inhibition due to high daytime temperatures is determined as per the original description of DOWNCAST (Jesperson & Sutton 1987). Conidia are predicted to disperse with the onset of morning once VPD has exceeded 0.6 hPa. Infections that occur the same morning as conidium release are predicted once the cumulative infection rate has reached or exceeded 2.3. If infection does not occur in the same morning as conidium release then infection is predicted once cumulative infection rate has reached, or exceeded, 3.3 to account for the additional time period required for rehydration of conidia and the reduction in viability due to age. Infection rates are calculated hourly based on temperature during the leaf wetness period. If leaf wetness ends prior to infection then the cumulative infection rate is reset to zero. As with sporulation this cumulative infection rate is analogous with the length of time required for infection under optimum temperatures, used in DOWNCAST. The proportion of conidia infecting is calculated based on the cumulative infection rate. For same morning

infections, 50 % of conidia produced are assumed to have dispersed, and of those one third are assumed available for infection. Following this 50 %, of conidia produced are assumed to survive the morning period, and the number of conidia present is reduced by 50 % for each subsequent 24 hour period until the age limit of 72 hours has been reached. Unlike DOWNCAST and ONIMIL, ZWIPERO does not account for conidium death due to slow rates of dew deposition. The relative number of conidia infecting is the product of the number of conidia available for infection and the proportion of infecting conidia.

An existing problem with DOWNCAST, ONIMIL and ZWIPERO is that they only provide relative measures of sporulation, as no account is taken of the level of initial inoculum within a crop, or if inoculum is present. Predictive models that do take into account initial inoculum have been developed for a few plant diseases. For example, the forecasting service provided by the North American Plant Disease Forecast Centre (NAPDFC) predicts the outbreak of tobacco blue mould in the United States over a regional scale. The model is principally based upon the weather conditions at the sites of known incidence, the predicted wind patterns for the next 48 hours and the weather conditions at sites free of the disease (Main *et al.* 1998). If the weather conditions are favourable at the sites of known incidence for sporulation, the wind patterns are used to predict the direction of conidium spread. If conditions are favourable for infection at sites along the path of predicted spread then infection at these sites is predicted to take place.

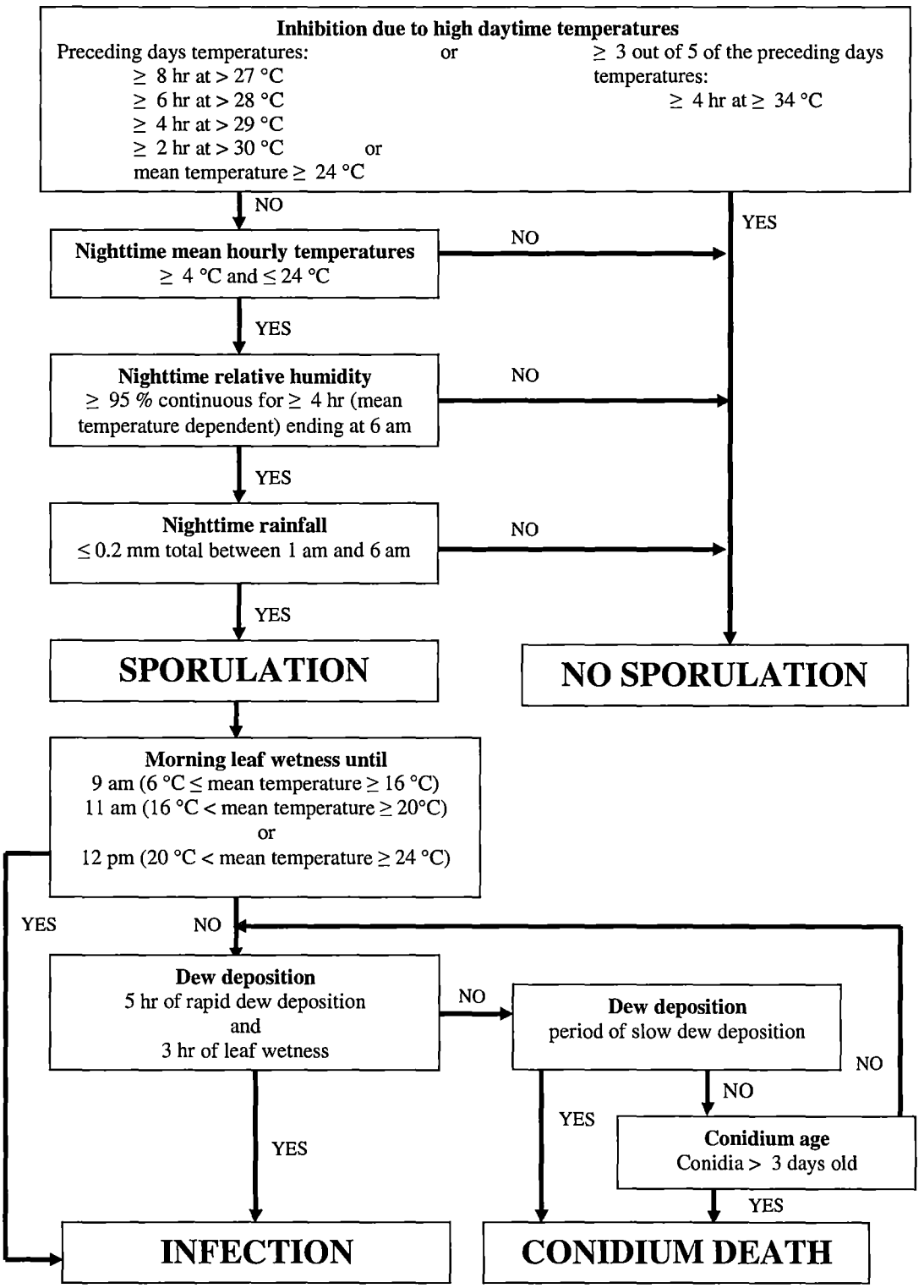


Fig. 2.10 Diagrammatic representation of the onion downy mildew forecaster, DOWNCAST, as described by Jesperson and Sutton (1987), and modified by de Visser (1998). Nighttime is considered to be the hours 20:00 to 6:00. Daytime is considered to be the hours 6:00 to 20:00.

2.2.5. Current control

The current methods utilised by the poppy industry in Tasmania to control downy mildew are based around either eradicant and/or protectant fungicide regimes to prevent the build up of downy mildew of crops over a given season. Cultural control methods such as crop rotations of three years, stubble burning, deep ploughing and control of volunteers are also used to prevent the build-up of downy mildew inoculum within paddocks.

Fungicides are currently applied in Tasmania on a calender-based schedule for control of downy mildew of poppy. Industry recommendations are based on the perceived disease pressure on a crop. Crops subjected to low disease pressures are sprayed with a recommended schedule of four sprays of the protective fungicide mancozeb or formulations containing phosphonic acid. Crops under high disease pressures are recommended to have an initial protective spray, followed by a limited number of sprays with one of the eradicant fungicides containing metalaxyl or dimethomorph.

Several problems exist with this reliance on fungicides as the primary means of control of poppy downy mildew. The dangers of widespread chemical pesticide use to human health and the natural environment have been well documented in recent years.

Fungicide application has been found to have some phytotoxic effects on poppy plants. Studies in India have shown that seed treatments with both metalaxyl (Doshi & Thakore 1995) and dithiocarbamates (Cheema *et al.* 1987), which include mancozeb, to control downy mildew infection in poppy seeds reduced the

germinability of seeds when stored following treatment. Metalaxyl applications have also been found to reduce the root length of poppy plants (Doshi & Thakore 1995), which may lead to reduced vigour of the plants.

The development of resistance to particular fungicides is another potential problem associated with the reliance on chemical control of *P. arborescens*. The continued use of a single fungicide type is well known to allow development of resistance to that chemical. No records of *P. arborescens* having developed resistance to specific fungicides currently exist. However, *P. parasitica* (Moss *et al.* 1994) and *P. viciae* (Falloon *et al.* 2000) were observed to have developed resistance to the phenylamide fungicides, which include metalaxyl. Falloon *et al.* (2000) also noted that for *P. viciae* at least, this resistance appears to be a stable characteristic in the population. It is therefore possible that *P. arborescens* may develop phenylamide resistance sometime in the future if metalaxyl is used successively in spray regimes. The introduction of dimethomorph as an alternative to metalaxyl has reduced the potential for this to occur. However, it should be noted that protectant fungicides, while less prone to the development of resistance, cannot be relied upon as the sole means of control for downy mildew outbreaks when the initial inoculum is plentiful or when the oomycete is capable of rapid spread (Palti & Rotem 1981).

To protect against the development of fungicide resistance, it would be preferable to integrate complementary control practices within chemical control programs (Palti & Rotem 1981). The use of cultural control methods such as microclimate modification and crop hygiene are two such practices (Palti & Rotem 1981). At present, the cultural control involves three year crop rotations, the control of volunteer poppies, the burning of crop stubble and deep ploughing of crop residues.

The period of three years recommended is based upon the work of Yossifovitch (1929), Kothari and Prasad (1970) and Alavi (1975). The use of disease forecasting systems can also reduce the need for chemical application. However, the undulating topography of Tasmania leads to very localised micro-climatic conditions between and within fields, which makes forecasting a very complex and difficult process.

2.3. Detection of downy mildew

The ability to detect downy mildew inoculum in plant material and environmental samples is critical for studies of the spread of the disease. Often these studies require detection of inoculum in seed and planting material, or from airborne or soil samples. While downy mildew infection is relatively simple to detect in symptomatic host plants, detection of the presence of inoculum in the absence of symptoms is more difficult. The obligate nature of *Peronospora* species means that artificial culturing techniques, such as selective media, cannot be used for detection of inoculum. In addition, the absence of the development of pathogen specific monoclonal antibodies, which is time consuming and expensive, prevents the use of serological techniques, such as enzyme-linked immunosorbent assays (ELISA; Clark & Adams 1977). Detecting inoculum in air, water or soil samples is further complicated by the near identical morphology of *Peronospora* spp. making species level determination using microscopic examination difficult. Microscopic examination is less of a problem with plant material as the limited host ranges of the various *Peronospora* spp. reduces the likelihood of misclassification. Despite these issues several different techniques still exist for the detection and diagnosis of downy mildew.

2.3.1. Biological detection

The simplest means of detecting downy mildew inoculum in planting material is to simply sow the seed or cuttings in conditions where infection from outside sources are prevented and after an adequate period of time examine for the presence of symptoms of infection. With downy mildew species infection is usually confirmed by placing seedlings in moist environments to promote sporulation (Inaba *et al.*

1983; Smith & Price 1997), which can then be observed visually. A limitation of seed germination is the length of time required for germination to occur.

The use of squashing, or crushing, techniques in conjunction with staining procedures can be used to detect the presence of inoculum in seed (Sugha *et al.* 1996; Zimmer *et al.* 1992). This is analogous to the use of histological clearing and staining techniques for plant tissues. The advantage of this form of microscopic observation is that it allows the location of inoculum within the host tissue to be determined. A disadvantage of staining and clearing techniques is the reliance upon morphological features for species determination. While the extraction of inoculum such as oospores from host material reduces the likelihood of other downy mildew species being present, confusion may still occur through the presence of oomycete species with similar morphologies, such as *Phytophthora* spp.

Another alternative means of detecting downy mildew infestation of plant material, such as seed, is through the seed washing test (Inaba *et al.* 1983; Pathak *et al.* 1978; Shetty *et al.* 1978). This involves the shaking of known quantities of seeds in sterile distilled water, decanting the resultant suspension and concentrating via centrifugation. Inoculum concentrations can then be determined by microscopic observation. This provides a quick and simple means of testing seed for inoculum (Inaba *et al.* 1983; Nali 1998). However, this test is disadvantaged by its inability to detect inoculum internal to the seed that cannot be dislodged by shaking (Sugha *et al.* 1996). A further expansion on this technique that does account for the presence of internal inoculum is the method of van der Gaag and Frinking (1993). In this method plant material is ground in sterile distilled water, sonicated to loosen inoculum from the ground material and sieved to collect the inoculum material. Again, inoculum

levels are determined by microscopic observation. Both of these techniques can incorporate the use tetrazolium salts as a stain for inoculum viability (Shetty *et al.* 1978; van der Gaag 1994). As before, the use of morphological features has the potential to lead to erroneous identifications. With all these techniques, sufficient sample sizes should be used to ensure adequate detection of inoculum and disease transmission, as instances of low rates of infection from seed-borne inoculum are common for downy mildew pathogens (Inaba *et al.* 1983; Vishunavat & Kolte 1993).

The use of healthy trap plants to detect the presence of environmental inoculum provides an alternative to traditional mechanical spore traps when dealing with plant pathogens. Most mechanical spore traps, such as Hirst's volumetric spore trap (Hirst 1952), rely on microscopic examination of the trapped material. Trap plants remove the ambiguity of downy mildew species determination based on morphology as the limited host ranges of downy mildews ensure that trap plants will only be infected by the spores of the desired mildew species. Trap plants may have the added advantage of being a more biologically accurate measure of inoculum as only viable inoculum under favourable conditions would be detected. It is this biological accuracy that leads to the frequent use of trap plants in studies of the effect of climate on disease progression (de Visser 1998; Fitz Gerald & O'Brien 1994). However, the use of trap plants has the disadvantage that it does not lead to direct quantification of inoculum levels. In addition, the latent period between trapping and the onset of symptom development, which for downy mildews can be 7 to 14 days (Hildebrand & Sutton 1984c; Viranyi 1975), is a constraint when rapid detection is required.

2.3.2. Molecular detection

The advent of the molecular technology for the detection of pathogens now provides an alternative to the use of biological methodologies. One of the key advantages of molecular detection is that tests can detect individual species based on DNA sequence which removes many of the ambiguities associated with detection based on morphology. Another advantage of molecular detection is the relative speed of the process compared to other detection techniques, with sample preparation and subsequent detection often able to be accomplished within a single day.

The basis for the majority of molecular detection techniques is the polymerase chain reaction (PCR). PCR uses short lengths of single stranded DNA (termed primers) to initiate the amplification of specific regions of the target species DNA (Saiki 1990), which can then be visualised through the use of gel electrophoresis. The sequence of these primers can be modified depending on the pathogen and position within the pathogen's genome to be amplified. Thus selection of the correct primer sequences can enable the detection of individual species. To design species specific primers the DNA sequence of the target region must first be known to allow comparison with other species and identification of unique DNA sequences for primer targeting. The most common target region for amplification in the downy mildew and related species are the internal transcribed spacer (ITS) regions of the ribosomal DNA, as these have been shown to contain enough sequence variability to allow species level determination (Constantinescu & Fatehi 2002; Cooke *et al.* 2000b; Crawford *et al.* 1996). The characterisation of universal primers for the ITS region (White *et al.* 1990) now allows easy amplification of this region which can then be sequenced. Within the genus *Peronospora*, species specific detection using primers targeting the

ITS region has now been developed for *P. sparsa* (Aegerter *et al.* 2002; Lindqvist *et al.* 1998).

An alternative means of developing PCR primers for species specific detection is through the use of randomly amplified polymorphic DNA (RAPD) markers (Wiglesworth *et al.* 1994). Short primers that promote the random amplication of the genomic DNA of the target species are used for PCR amplification. The resultant products can then be compared to those from other species and products unique to the target species can be sequenced for primer development. This method of primer development has been conducted for *P. tabacina* (Wiglesworth *et al.* 1994). RAPD markers can also be used to detect variation within the genome of species, which may be due to the presence of pathotypes, as has occurred for *P. parasitica* (Tham *et al.* 1994).

Where species specific primers cannot be readily developed, detection may be conducted through the use of PCR restriction fragment length polymorphism (PCR-RFLP). The digestion of amplicons from universal or generic PCR's with restriction enzymes that target specific sequences within the amplicon can result in product patterns specific to individual species, dependant upon enzyme selection (Matsumoto *et al.* 1997; Nielsen *et al.* 2002). The additional step involved in PCR-RFLP detection does mean an increase in the time of detection procedures relative to detection using specific primers.

A disadvantage of most forms of molecular detection are their inability to quantify the levels of inoculum present in a given sample, with most tests providing simple positive/negative results. The advent of molecular quantification techniques, such as

real-time PCR (Bohm *et al.* 1999), now make up for this shortcoming. Another disadvantage of molecular detection is that it cannot distinguish between living viable inoculum and residual DNA from non-viable propagules.

2.4. Characterising epidemic development

2.4.1. Spatial analysis

Knowledge of the spatial pattern of epidemics is advantageous for plant disease epidemiologists as it can provide information on the relative importance of primary and secondary inoculum, the mechanisms of pathogen dispersal, and the effects of environmental factors on epidemics. In addition, understanding the spatial nature of epidemics is vital for the designing of survey methods for accurate disease measurement (Parker *et al.* 1997b). The aim of this review is to provide an overview of the more common spatial analysis techniques used in plant pathology, with their relative advantages and disadvantages. It should be noted that the selection of spatial analysis techniques in plant pathological studies typically vary from those employed in ecological studies due to regular spacing of sowings employed in agronomic situations, conflicting with the assumption of random distribution of individuals employed by many ecological models (Gray *et al.* 1986).

Due to the differing data requirements of spatial analysis techniques the selection of a particular technique for assessment is usually based on the practicality of sampling techniques for the pathosystem in question. When assessing disease epidemics, choices must be made whether to assess disease incidence (the proportion of diseased plants) or disease severity (the proportion of plant organs diseased or sometimes number of diseased lesions) or both (Campbell & Madden 1990). Incidence is much simpler and quicker to measure, but contains less information than severity (Campbell & Madden 1990). Severity may be a more important measure than incidence in terms of disease management and population dynamics (Campbell & Madden 1990), but is also less accurate to measure (Campbell & Madden 1990;

Parker *et al.* 1997b). The choice of sampling pattern must also be made. Sampling may be intensive, where every plant with the study area is assessed, or sparse, where a proportion of sampling units are assessed (Diggle 1983). Intensive sampling, which usually takes the form of a lattice arrangement, has the advantage of increased information and accuracy, but may not be practical for many pathosystems. If a sparse sampling plan is adopted, a further choice must be made between sampling from contiguous quadrats or from transects (Campbell & Madden 1990). Again, quadrat data contain greater information, while transects have the advantages of speed and simplicity (Campbell & Madden 1990).

Spatial analysis techniques can be divided into two broad categories; point-pattern and correlation type analyses (Upton & Fingleton 1985). Under point-pattern analyses, 'points' are discrete sampling units consisting of individual diseased plants or collections of individuals within a discrete location (Madden 1989). Counts of individuals at locations or the distances between locations can then be used to determine the spatial distribution (Madden 1989). Point-pattern analyses are independent of the spatial location of points (Madden 1989). The alternative to point-pattern analyses are correlation analyses, which compare the values of spatially referenced sampling units (Madden 1989; Upton & Fingleton 1985). As such point-pattern analyses are concerned with spatial processes at the scale of sampling unit and below, whilst spatial autocorrelation deals with the scale of sampling unit and above (Turechek & Madden 1999b).

Table 2.1 Summary of the characteristics of common spatial analysis techniques employed in plant pathology.

Test	Point-pattern or correlation	Data types	Sampling type	Visual representation?	Anisotropy detecting?[1]
Distribution fitting	point-pattern	binary and discrete	transect and quadrat	no	no
Taylor's Power Law	point-pattern	binary and discrete	transect and quadrat	no	no
Spatial autocorrelation	correlation	binary, discrete and continuous	lattice, transect and quadrat	yes	yes
Geostatistics	correlation	binary, discrete and continuous	lattice, transect and quadrat	yes	yes
Ordinary runs	correlation	Binary	lattice and transect	no	yes
Median runs	correlation	discrete and continuous	transect and quadrat	no	yes
Join counts	correlation	Binary	lattice	no	yes
SADIE	correlation	binary and discrete	lattice, transect and quadrat	yes	yes
Disease gradients	correlation	binary, discrete and continuous	lattice, transect and quadrat	yes	yes

[1]Able to differentiate the spatial patterns of different directions

Typical point-pattern analyses that are utilised for spatial analysis of plant diseases include distribution fitting and Taylor's Power Law analysis (Table 2.1). Distribution fitting involves the fitting of standard distributions to the observed distributions of values within discrete sampling units (Hughes & Madden 1993). For disease incidence assessments the status of individual plants is either positive or negative, therefore the data collected is binary in nature. As such, randomly distributed disease counts have a typical binomial distribution, with each sampling unit having an equal probability of disease at each point, π (Hughes & Madden 1993). Under a clustered disease pattern, disease incidence varies from sampling unit to unit, and has a beta-binomial distribution which is defined by two parameters, the expected probability of disease for each point (π), and the variance of that expected probability, θ (Hughes & Madden 1993). The value of θ is a measure of the heterogeneity of the data. As θ approaches 0, the beta-binomial becomes equal to the binomial distribution. Therefore, under distribution fitting, both the binomial and beta-binomial distributions are fitted to the observed data and the distribution of best fit is determined under a null hypothesis that the beta-binomial does not provide a better fit to the data than the binomial distribution (Hughes & Madden 1993). Rejection of the null hypothesis is evidence for overdispersion, or a clustered disease pattern, against failure to reject the null hypothesis which is evidence of a random disease pattern (Hughes & Madden 1993). An index of dispersion (D) is calculated by finding the ratio of the observed variance to the theoretical variance under the binomial distribution (Madden & Hughes 1994). High values of D indicate increased levels of clustering. This is then used to test for the distribution of best fit. Fitting the binomial and beta-binomial distributions is only suitable for binary data and cannot be used for severity data. However, when severity data is in the form of discrete counts of lesion numbers per sampling unit it can be fitted to the Poisson

(random) and negative binomial (clustered) distributions (Madden & Hughes 1995). As with binary data, indices of dispersion can be calculated to allow determination of the model of best fit, the most common of which is the variance to mean ratio, *VM* (Ludwig & Reynolds 1988). Under the Poisson distribution the ratio of variance to mean is equal to one, while significantly higher values of *VM* are evidence for disease clustering (Ludwig & Reynolds 1988; Madden 1989). Analysis of this type is not used for severity data in the form of the continuous variable of percentage leaf area diseased. Distribution fitting is designed for quadrat or transect sampling patterns and is not suitable for intensively mapped, individual incidence scores, unless these are first converted to quadrat data.

A second point-pattern analysis that is commonly used in plant pathology is Taylor's Power Law analysis, which is used to assess heterogeneity of multiple data sets. Like distribution fitting, power law analysis can be used for both count data and binary incidence data (Hughes & Madden 1992; Madden & Hughes 1995). The original form of Taylor's Power Law can be used to analyse multiple sets of disease count data by plotting the logarithms of variances of each set against the logarithms of the respective means. The slope and intercept of the resultant linear regression is then used as a measure of clustering (Hughes & Madden 1992). Hughes and Madden (1992) developed a binary form of the power law for the analysis of disease incidence sets. In this case, the logarithm of the observed variance is plotted against the logarithm of the theoretical variance under the binomial distribution (Hughes & Madden 1992). As is the case with distribution fitting, no analysis of this type is available for the assessment of continuous data sets and it cannot be used with intensively mapped data sets, unless converted to quadrat data.

Correlation type analyses that are typically employed by plant pathologists include spatial autocorrelation, geostatistical methods, the runs analyses, spatial analysis by distance indices (SADIE) and disease gradients (Table 2.1). Spatial autocorrelation analysis uses the assumption that sampling units of increasing proximity will have values of increasing correlation between them, i.e., autocorrelation (Campbell & Madden 1990; Hughes *et al.* 1997). A variety of correlation statistics can be calculated, including Moran's *I* and Geary's *c* (Campbell & Madden 1990; Upton & Fingleton 1985). Spatial autocorrelation can be calculated in the first order (i.e., adjacent sampling units, a lag of 1) or over a set number of lags, e.g. a lag of 2 indicates the autocorrelation between two sampling units separated by a single sampling unit (Campbell & Madden 1990; Upton & Fingleton 1985). This allows the determination of the extent of clustering that may occur within a study area, and is typically represented as a correlogram where the correlation statistic is plotted against the lag distance of separation (Campbell & Madden 1990; Madden 1989; Upton & Fingleton 1985). In addition, the uneven clustering of spatial pattern based on direction (anisotropy) can be detected through the selection of the definition of proximity. Sampling unit proximity may be defined as down rows, across rows, both down and across rows ('Rook' definition), diagonally ('Bishop' definition), a combination of the Rook and Bishop definition ('Queen' definition), or a square proximity (Fig. 2.10; Campbell & Madden 1990; Madden 1989; Upton & Fingleton 1985). Binary, discrete and continuous data sets can all be analysed by spatial autocorrelation (Campbell & Madden 1990), as can both transect and quadrat data.

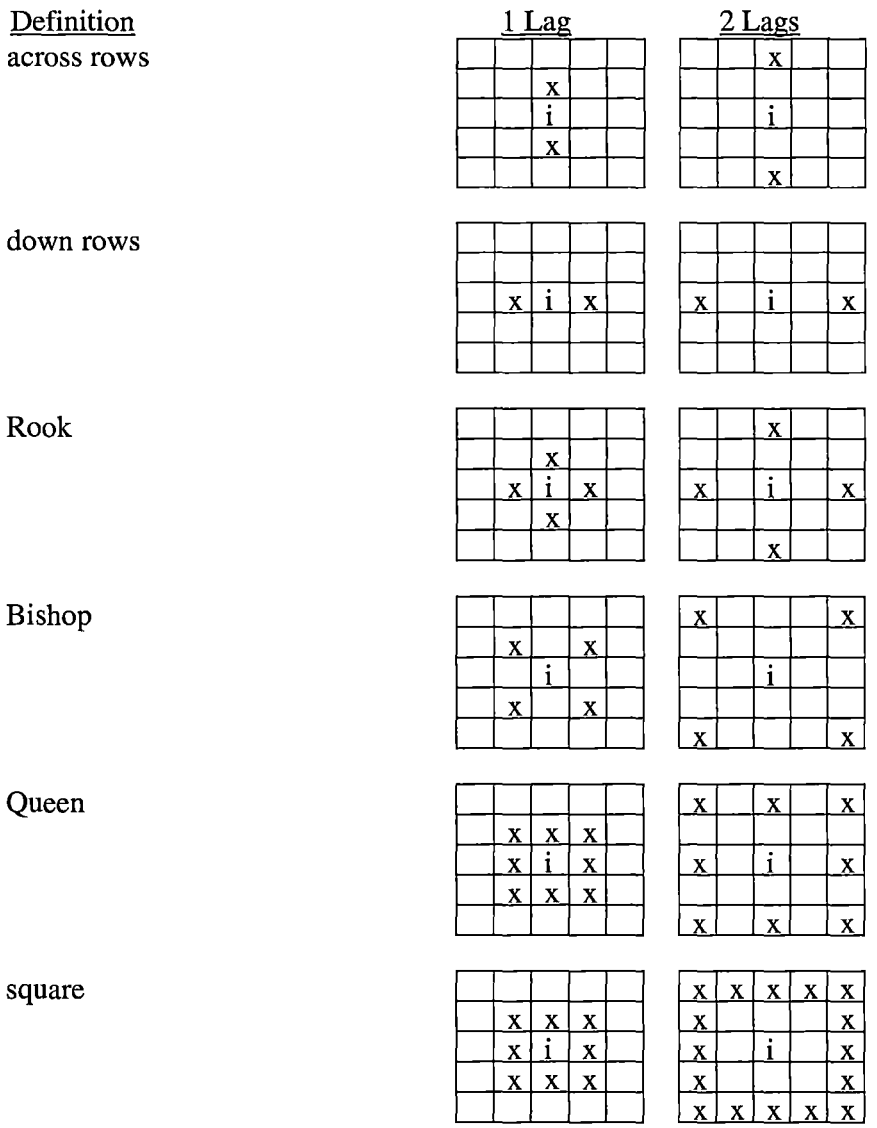


Fig. 2.11 Definitions of proximity used in spatial autocorrelation analysis. Symbol i indicates sampling unit of interest; symbols x indicate proximate sampling units under the specific definition.

Closely related to spatial autocorrelation analysis are geostatistics. Geostatistics use the assumption that the difference between sampling units is a function of distance of separation (Chellemi *et al.* 1988; Hughes *et al.* 1997). The basis for geostatistics is the semivariogram, the mirror image of the spatial autocorrelogram (Hughes *et al.* 1997). The semivariogram is a plot of the semivariance ($\gamma(d)$), a measure of the expected difference between the values of all sampling units separated by a given distance (Chellemi *et al.* 1988), against the distance of separation. Three key

features of the semivariogram are the nugget, the sill and the range. The nugget is the intercept of semivariogram at the Y-axis and is a measure of the effect of sampling error and other random factors on $\gamma(d)$ (Dandurand *et al.* 1995; Hughes *et al.* 1997). The sill is the value of $\gamma(d)$ where the spatial dependance is lost, while the range is the distance at which this occurs (Dandurand *et al.* 1995; Hughes *et al.* 1997). Geostatistics can further be expanded through the process of kriging to provide estimates for unsampled areas within the study area (Hughes *et al.* 1997; Nelson *et al.* 1999). Geostatistics is compatible with binary, discrete and continuous data sets as well as both intensive and sparse sampling patterns (Hughes *et al.* 1997). Anisotropy can also be examined with geostatistics by constructing sets of semivariograms of distinct orientation (Larkin *et al.* 1995; Nelson *et al.* 1994). Geostatistical methods are also compatible with global information systems (GIS), which allows the collation of climatic, crop and other factors, with disease measurements to detect relationships between these factors (Nelson *et al.* 1994; Nelson *et al.* 1999).

Another type of correlation analysis is the runs 'family' of analyses, including doublet, ordinary runs, median runs, and join counts. With the exception of median runs, runs analyses are designed for intensively sampled disease incidence data sets. Doublet analysis uses the principle of two adjacent diseased plants within a row counted as one doublet, while three adjacent disease plants are counted as two doublets (van der Plank 1946). The observed number of doublets is then compared to the expected number of doublets under the null hypothesis of no clustering, with observed values greater than the expected being evidence for clustering (van der Plank 1946). An alternative to doublet analysis is ordinary runs, where a run is defined as one or more adjacent sampling units with the same disease score bordered

by sampling units with differing disease scores (Gibbons 1985; Madden *et al.* 1982). The observed number of runs is then compared to the expected number of runs, with significantly less runs than expected providing evidence of clustering (Gibbons 1985; Madden *et al.* 1982). The suitability of both doublets and ordinary runs for the analysis of spatial pattern has been assessed by Madden *et al.* (1982), with doublet analysis providing unsatisfactory results and ordinary runs determined to be the best test for the randomness of spatial pattern. It was found that doublet analysis provided greater than 5 % misclassifications under both random and clustered simulated disease patterns, whilst ordinary runs provided less than 5 % misclassifications under both sets of simulations (Madden *et al.* 1982). An extension of ordinary runs, median runs, enables the use of this technique for non-binary data, from either intensively or sparsely mapped data (Gibbons 1985). Under median runs the median value of the sampling units is determined and sampling units are then reassigned scores of 1, for values greater than the median, or 0, for values equal to or less than the median (Gibbons 1985). From this point, median runs is treated the same as ordinary runs. One advantage of both ordinary and median runs is that analysis of two dimensional lattices can be conducted both down and across, thus providing some information on the presence of anisotropy. A final analysis that is closely related to runs analysis is join counts analysis (Upton & Fingleton 1985). While ordinary and median runs analysis convert two dimensional lattices into one dimensional structures, join counts maintains the two dimensional structure of the lattice (Hughes *et al.* 1997). Joins can be defined as contiguous regions of two healthy plants (H-H), two diseased plants (D-D) or one healthy plant and one diseased plant (H-D). The observed number of joins is then compared to the expected number of joins under the null hypothesis. The two dimensional nature of join counts allows for comparison between plants in multiple directions

simultaneously (Upton & Fingleton 1985). As with spatial autocorrelation analysis, comparisons can detect anisotropy via the selected definition of proximity (Upton & Fingleton 1985), and the extent of clustering can be measured by defining contiguity over a series of lags (Pethybridge & Madden 2003).

SADIE is correlation type analysis designed for discrete count data, but differs from other correlation analyses in that it uses data in the form of point-patterns and is therefore conditioned upon the inherent heterogeneity within a data set (Perry 1995). Central is the concept of distance to regularity. SADIE uses a transportation algorithm to calculate the minimum total distance it is necessary for individuals within a data set to move to generate a completely regular spatial pattern (Perry 1995). An index of aggregation (I_a) is then calculated from the ratio of the observed distance to regularity to expected distance to regularity, which is equal to the mean of calculated distances to regularity under random reallocations of observed counts (Perry 1995). Values of I_a greater than 1 are evidence of clustering, while values equal to 1 are evidence of random patterns (Perry 1995). A second index of aggregation (J_a) can also be calculated under the reverse situation of distance to crowding, where the minimum distance to place all individuals in a single sampling unit is determined (Perry 1995). However, the power of this test is greatly reduced when more than one cluster is present within a data set (Perry 1995), and it is therefore not generally used. An added feature of SADIE is the ability to visually represent the spatial pattern of a data set through the use of red-blue cluster plots that use the clustering indices of the individual sampling units calculated during the determination of I_a (Perry *et al.* 1999). While SADIE is principally designed for count data, it can be used to analyse presence/absence data from intensively mapped data sets (Perry 1998), but is unable to analyse continuous data sets without

transformation. SADIE is also able to analyse irregularly shaped sampling areas (Perry 1995). SADIE may be a more appropriate analysis technique than spatial autocorrelation and geostatistics for the analysis of binary or count data due to their discrete nature and the frequent occurrence of zero's (Turechek & Madden 1999a).

In controlled disease studies where only one disease focus of known position is present within a field (e.g. created by artificial inoculation), the spatial spread of disease can be measured with the use of disease gradients (Campbell & Madden 1990; van der Plank 1967). The change in disease levels is compared over the distance of separation from the disease focus to indicate the extent of disease spread. Typically empirical gradient models such as the power law and exponential models are fitted to the observed data to characterise the extent of spread (van der Plank 1967). Disease gradients can also be used to detect anisotropy by the construction of unidirectional gradients (Gottwald 1995; Parker *et al.* 1997a), and are compatible with binary, discrete and continuous data sets.

As can be seen from the summaries provided here, different spatial analysis techniques have different strengths and weaknesses. It is therefore often best to use a combination of techniques, usually a point-pattern and a correlation analysis, to provide a more accurate assessment of spatial pattern (Hughes *et al.* 1997). It has also been noted that overall most spatial pattern assessment techniques provided similar conclusions and therefore it is more important to measure spatial pattern rather than argue which model to use (Keitt *et al.* 2002).

2.4.2. Spatial association

An extension of the analysis of spatial pattern that is often employed is analysis of spatial association between two or more species within the same sampling area. This is a measure of the affinity, or lack there of, for coexistence between these species (Ludwig & Reynolds 1988). Species may exhibit association with one or other because of common habitual requirements, an attraction exists between one or more of the species, or in the case of plant pathogens, species may have related modes of pathogen entry or dispersal (Ludwig & Reynolds 1988; Schluter 1984). Dissociations may occur due to the converse of the above, or due to antagonistic processes such as the release of toxins or antibiotics (Pethybridge & Turechek 2003). Two forms of spatial association can be measured, co-occurrence and co-variation. Co-occurrence is the tendency of species to occupy the same habitat, while co-variation refers to the tendency of the density of one species to increase with the increase in another (Turechek & Madden 2000). As can be seen by these definitions, differences occur between the properties of these two analysis types. Co-occurrence is assessed using binary data for the individual species, and when the association between two species is assessed overall sampling unit scores can only take on one of four states; both species present, only species A present, only species B present, or both species absent (Pethybridge & Turechek 2003). Co-variation, conversely, uses data in the form of discrete counts or continuous density measurements. It is noted that spatial association analysis is best used as a simple descriptor of patterns, which are then used to generate explanatory hypotheses for further testing (Nelson & Campbell 1992; Schluter 1984).

Typical co-occurrence analyses that are employed by plant pathologists include the Jaccard similarity index, the Ochiai index, the Dice index and the binary form of

Robson's variance test (Table 2.2). The Jaccard similarity, Ochiai and Dice indices are all designed for detecting association between species pairs, based upon two by two contingency tables (Ludwig & Reynolds 1988). All three indices compare the number of sampling units containing both species to a function of the total number of sampling units containing at least one species, with values of 0 indicating complete dissociation and 1 indicating complete association (Ludwig & Reynolds 1988). Studies of the strength of these tests have shown that the Jaccard index is generally unbiased, but the Dice index tends to underestimate the true population values at small sample sizes (Ludwig & Reynolds 1988). In the tests referenced by Ludwig and Reynolds (1988), no testing of the Ochiai index occurred. Two key issues limit the use of these indices in plant pathological studies. Firstly, it is assumed by all three indices that the individual species populations are randomly distributed in space, which is often not the case in disease epidemics. The spatial pattern of individual species can have a strong influence on the levels of association detected (Perry & Dixon 2002), and therefore the assumption of random patterns is potentially erroneous. Secondly, the sampling distributions of these indices is unknown, therefore the value of each index that indicates independence is unknown and no standard method of statistically testing for association is available (Pethybridge & Turechek 2003; Turechek & Madden 2000). Recent work by Turechek and Madden (2000) has led to the development of a Jackknife procedure for the estimation of standard errors and a randomisation test to calculate the expected Jaccard index value under the null hypothesis of no association, which accounts for the second of these issues.

The Robson's variance test is designed to detect the co-occurrence between more than two species within a study area. The test finds the ratio of the observed variance

in the total number of species in sampling units to the expected variance under the null hypothesis of no association (Schluter 1984). Multiplication of this ratio by the total number of sampling units (N) provides a test statistic that is assumed to have a χ^2 distribution with N degrees of freedom under the null hypothesis (Schluter 1984). As with the previous methods of assessing co-occurrence mentioned here, the variance test is independent of the spatial pattern of the individual component species. Another fault with the variance test is that the results are susceptible to the occurrence of association between some component species and dissociation between others leading to an overall failure to reject the null hypothesis (Schluter 1984).

Table 2.2 Summary of the characteristics of common spatial association techniques employed in plant pathology.

Test	Co-occurrence or co-variation	# component species	Data types	Accounts for spatial pattern?
Jaccard similarity index	co-occurrence	2	binary	no
Ochiai index	co-occurrence	2	binary	no
Dice index	co-occurrence	2	binary	no
Robson's variance test	co-occurrence	> 2	binary	no
	co-variation	> 2	discrete and continuous	no
Pearson's correlation	co-variation	2	discrete and continuous	no
SADIE	co-variation	2	binary and discrete	yes
Spearman's rank correlation	co-variation	2	binary, discrete and continuous	no

Typical co-variation analysis techniques that are employed by plant pathologists include Pearson's correlation, SADIE, Spearman's rank correlation co-efficient, and the density form of Robson's variance test (Table 2.2.). Pearson's correlation co-efficient, *r*, can be used to assess co-variation between samples (Ludwig & Reynolds 1988), independent of spatial pattern, in the same manner that correlations can be made between many other non-spatial variables (Glover & Mitchell 2002; Steel & Torrie 1980). Using discrete or continuous data sets, Pearson's correlation provides an easy test of the relationship between species. However, the lack of account taken for spatial pattern may lead to inaccurate results in the face of a non-random spatial pattern in one, or both, component species (Perry & Dixon 2002). Use of *r* may also be limited by the assumption of a bivariate normal distribution, where both species have to be drawn from a normal distribution (Ludwig & Reynolds 1988).

An alternative, but related, measure of co-variation can be obtained through the use of SADIE (Perry & Dixon 2002; Winder *et al.* 2001). Recently a new method of assessing spatial association using SADIE has been described based on the clustering indices for individual sampling units, which are output as part of the SADIE analysis of spatial pattern (Perry & Dixon 2002; Winder *et al.* 2001). The clustering index values for each species at each individual sampling unit are then correlated to give a measure of local association, with the mean of these local associations providing an overall measure of spatial association, *X* (Perry & Dixon 2002; Winder *et al.* 2001). This in effect is the Pearson's correlation of these clustering indices and is therefore closely related to *r* (Perry & Dixon 2002). The differences between *X* and *r* are due to SADIE's use of the clustering indices rather than the observed sampling unit values, while the clustering units, are based in part on the observed values, they also

include an inherent measure of spatial pattern (Perry & Dixon 2002). In this case, the data limitations are those of the assessment of spatial pattern by SADIE.

If a bivariate normal distribution cannot be assumed then an alternative measure of co-variation can be assessed using the non-parametric Spearman's rank correlation (Ludwig & Reynolds 1988; Pethybridge & Turechek 2003; Turechek & Madden 2000). Under this test the individual sampling unit values for each species are ranked and the ranks of the component species are then compared at each sampling unit to provide an overall correlation co-efficient, r_s (Ludwig & Reynolds 1988). Under association, high ranks will coincide at the same sampling units, while under dissociation the high ranks of one species will coincide with the low ranks of the other species (Ludwig & Reynolds 1988). Binary, discrete and continuous data are all compatible with r_s. However, with discrete data the non-parametric nature of the co-efficient ensures that no measure of the heterogeneity of the original data sets is included in the analysis and results may therefore differ from those of SADIE (Pethybridge & Turechek 2003).

When assessing the co-variation of multiple (> 2) species a second form of Robson's variance test is available (Schluter 1984). In this test, alternative observed and expected variances are calculated based on the non-binary nature of the data sets (Schluter 1984). All other properties of this test are the same as that of the binary form of Robson's variance test.

2.4.3. Temporal and spatiotemporal analysis

In addition to its spatial component, epidemic development also has a temporal component that should be considered during analysis. To assess this component temporal and spatiotemporal analysis techniques have been developed.

Temporal analysis is typically conducted through the fitting of standard disease progress curves (Fig. 2.12) to observed data (Madden 1980; Nutter 1997; Parker *et al.* 1997b). Disease epidemics are typically described as monocyclic, van der Plank's simple interest disease, or polycyclic, van der Plank's compound interest disease (van der Plank 1963). Under monocyclic disease progression only one generation occurs in any given season, thus the only source of inoculum for an epidemic is external primary inoculum (van der Plank 1963). Polycyclic diseases produce more than one generation in a given season, therefore in addition to a primary inoculum source, secondary inoculum is generated within the crop following initial infection (van der Plank 1963). Monocyclic and polycyclic disease can be characterised based on disease progress curves. Monocyclic diseases are typically described by a monomolecular disease progress curve (Fig. 2.12A), under which the rate of spread is greatest at the point of initial infection and decreases from that point onwards (Madden 1980; Madden *et al.* 1987). Polycyclic diseases can be typically described by either the exponential, logistic or Gompertz models (Fig. 2.12B-D; Madden 1980; Madden *et al.* 1987). The exponential curve (Fig. 2.12B) describes the situation where the rate of infection is lowest at the time of initial infection and increases with increasing levels of secondary inoculum production over time (Madden 1980; Madden *et al.* 1987). The logistic curve (Fig. 2.12C) is an extension of the exponential curve where the rate of spread increases initially, but then becomes limited by reducing levels of available healthy host tissue (Madden 1980;

Madden *et al.* 1987). The Gompertz curve (Fig. 2.12D) has a similar shape to the logistic curve, but has a more rapid rate of increase in the rate of spread and a slower decline in the rate of spread (Madden 1980; Madden *et al.* 1987). However, it should be noted that not all polycyclic diseases can be described by these models (Madden 1980). Instances of a polycyclic disease dominated by primary inoculum have been recorded (Hau *et al.* 1995).

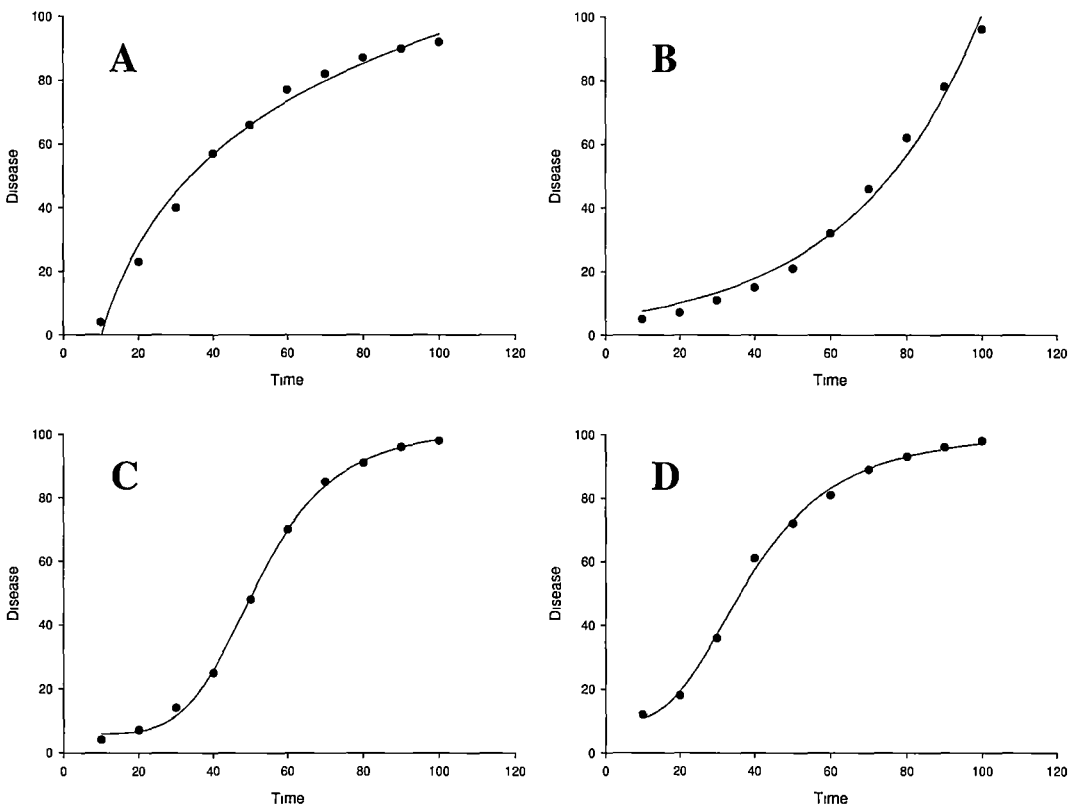


Fig. 2.12 Visual representation of the standard disease progress curves; A) monomolecular; B) exponential; C) logistic; D) Gompertz. Data source Nutter and Parker .

Spatiotemporal analysis combines the spatial and temporal components of epidemics into a single analysis. The most commonly used spatiotemporal analyses include directional disease progress curves, temporal disease gradients, spatiotemporal autocorrelation, Markov-chain Monte Carlo methods (MCMC), geostatistics and

SADIE (Table 2.3). It should be noted that while spatiotemporal analyses account for both the spatial and temporal dynamics of epidemics it is often best to use these in conjunction with spatial and temporal analysis techniques (Gibson 1997). Directional disease progress curves constitute the simplest means of measuring the spatiotemporal dynamics of an epidemic. A spatial component is added to disease progression analysis by dividing a study area into quadrats and disease progression is then characterised for each individually and overlayed on the same plot for comparison (Gottwald 1995; Parker *et al.* 1997b). The same means can be used to provide a temporal component to spatial analysis conducted with disease gradients. In this case, gradient analysis is conducted for several time periods over the season and the resultant disease gradients are overlayed to provide an indication of how disease spread has changed over time (Campbell & Madden 1990).

Table 2.3 Summary of the characteristics of the common spatiotemporal analysis techniques employed in plant pathology.

Test	Data type	Sampling type	Visual representation?
Directional disease progress curves	binary, discrete and continuous	quadrat	yes
Temporal disease gradients	binary, discrete and continuous	lattice, transect and quadrat	yes
Spatiotemporal autocorrelation	binary, discrete and continuous	lattice, transect and quadrat	yes
Geostatistics	binary, discrete and continuous	lattice, transect and quadrat	yes
SADIE	binary and discrete	lattice, transect and quadrat	yes
MCMC	binary	lattice	yes

Many other methods of spatiotemporal analysis develop as extensions of spatial analysis techniques. Spatiotemporal autocorrelation is an extension of spatial autocorrelation, under which disease is related to disease levels at the same location at the previous time period (Reynolds & Madden 1988; Reynolds *et al.* 1988). Spatiotemporal analysis can also be conducted using geostatical methods. This is conducted with the use of a two-variable semivariogram accounting for distance in both space and time (Stein *et al.* 1994). Analysis is then conducted for space and time by setting the distance of separation of one, then the other, to zero. Space-time kriging can also be conducted to estimate unsampled regions in space and time (Stein *et al.* 1994). An adaptation of the measurement of spatial association allows for the assessment of spatiotemporal dynamics using SADIE (Winder *et al.* 2001). Instead of assessing the association between two component species, SADIE can be used to assess the association between the spatial patterns of a disease over a pair of time periods. The presence of association or dissociation between the component time periods can then be used to formulate hypotheses as to the processes governing these relationships (Winder *et al.* 2001). Contour plotting of the local association values for sampling units provides a visual representation of regions of local association and dissociation within the study area (Perry & Dixon 2002; Winder *et al.* 2001).

The use of Markov-chain Monte Carlo stochastic modelling (MCMC) provides another means of spatiotemporal analysis (Gibson 1997). The key principle behind MCMC is the probability of a plant being infected in a given time being based on the distance of that plant from other infected plants in the previous time period (Gibson 1997; Gibson & Austin 1996). Two parameters, a_1 (which is transformed into b) and a_2, are estimated in MCMC to provide measures of the extent of primary and secondary spread respectively (Gibson 1997). A value of 0 for b indicates no

primary spread, while increasing values indicate increasing levels of primary spread. Likewise, a value of 0 for a_2 indicates no secondary spread within a field or secondary spread over large distances, such that it cannot be distinguished from primary spread. As a_2 increases, the distance that secondary spread occurs over decreases, with values of three or greater indicating spread to neighbouring plants only. This technique has been developed for the analysis of intensively mapped data in agronomic situations, and therefore requires a binary lattice form of data, with the distance between rows and columns assumed to be equal (Gibson 1997). The use of Markov-chains allows MCMC to account for missing values within the lattice (Gibson 1997). Contour plots of the confidence intervals of the parameters b and a_2 provides a visual representation of the extent of primary and secondary spread.

3. Identification of the downy mildew pathogen of poppy in Tasmania

3.1. Introduction

Five species from within the oomycete genus, *Peronospora*, have been recorded as downy mildew pathogens of members of the plant genus *Papaver*, these are *P. arborescens* (Berk.) Casp., *P. argemones* Gaum., *P. cristata* Tranz., *P. grisea* Ung. var. *minor* Casp., and *P. papaveris-pilosi* Viennot-Bourgin (Constantinescu 1991). However, the taxonomic status of these species is complicated. *Peronospora papaveris-pilosi* is considered an invalid record, whilst *P. grisea* var. *minor* is considered conspecific with *P. arborescens* (Constantinescu 1991). Conspecificity between *P. argemones* and *P. cristata* has also been argued based on the similarity of published conidium dimensions, and the older *P. cristata* taxon is preferred (Reid 1969). In the same study, *P. cristata* was differentiated from *P. arborescens* based on the latter's smaller average conidium dimension on *Meconopsis* spp. (Reid 1969). The differentiation of species based on morphological dimensions alone is problematic, as these can change with host genotype and environmental conditions (Hall 1996). A more accurate definition of species may be obtained through combining phylogenetic analyses of molecular characters with morphological dimensions (Hall 1996). Previous studies have shown the internal transcribed spacer (ITS) regions of ribosomal RNA genes (rDNA) to be useful for the differentiation of downy mildew species and related organisms at the species level (Constantinescu & Fatehi 2002; Cooke *et al.* 2000a; Crawford *et al.* 1996).

The host range of the two oomycete species in question also exhibits a degree of overlap. *Peronospora arborescens* has been recorded as a pathogen of the plant

species *Papaver rhoeas* L. (Berkeley 1846; Gustavsson 1959), *Pap. somniferum* (Behr 1956; Kothari & Prasad 1970; Yossifovitch 1929), *Pap. dubium* L. (Cotterill & Pascoe 1998; Cotton 1929; Gustavsson 1959), *Pap. argemone* L. (Cotton 1929), *Pap. nudicaule* L. (Alcock 1933; Cotterill & Pascoe 1998), *Pap. setigerum* L. (Behr 1956), *Pap. alpinum* L., *Pap. caucasicum* Bieb. (Francis 1981), *Argemone mexicana* L. (Maiti & Chattopadhyay 1986), and many species of *Meconopsis* (Alcock 1933; Cotton 1929; Reid 1969). *Peronospora cristata* has been recorded as infecting *Pap. hybridum* L. (Constantinescu 1991), *Pap. argemone* (Gaumann 1923; Gustavsson 1959), and *M. cambrica* Vig (Reid 1969). Of the known host species in Tasmania, *Pap. somniferum* is an introduced crop, *A. mexicana, Pap. rhoeas, Pap. dubium, Pap. argemone and Pap. hybridum* are introduced weeds of cultivation (Curtis 1993), and *Pap. nudicaule* (and *Pap. rhoeas*) are introduced ornamental species. As there is no known native host of poppy downy mildew in Tasmania, it is likely that the disease pathogen is also introduced. It should be noted that Kothari and Prasad (1970) tested *Pap. rhoeas* and *A. mexicana* and were unable to infect using isolates of *P. arborescens*, suggesting the host range of species may vary with pathotype.

The main objectives of this study were to determine the inter-, and intra-specific, relationships of the poppy downy mildew pathogen based on DNA sequence analysis of the ITS1, 5.8S gene and ITS2 region, and morphological dimensions. A secondary objective of this work was to develop polymerase chain reaction (PCR) primers for the specific detection of the Tasmanian poppy downy mildew pathogen.

3.2. Materials and methods

3.2.1. Sample collection

Leaf samples were collected from commercial poppy crops around Tasmania in field surveys (Table 3.1; Appendix I). Leaves were incubated in sealed trays containing damp tissue at 100 % relative humidity, and 12 °C in the dark for 12 hours to induce sporulation. Conidia were washed from leaves into centrifuge tubes with distilled water. Conidium suspensions were concentrated by centrifugation at 3000 *g* (r_{av} 139 mm) for 20 min at room temperature and the upper layer removed by pipetting and discarded. Spore concentrations were determined with a haemocytometer and adjusted to 10^4 spores.mL^{-1}. Slides were made by collecting conidia and conidiophores from leaves with cellotape and staining with 0.05 % aniline blue (50 % glycerol, 25 % lactic acid, 25 % distilled H_2O).

3.2.2. Morphological observations

The morphology of the available sexual structures of all specimens collected was observed visually at 1000x magnification under oil immersion with a Zeiss Axiolab compound microscope (Carl Zeiss, Oberkochen, Germany) with Achronplan objective lenses (Carl Zeiss, Oberkochen, Germany). Observations were compared to the published descriptions of *Peronospora* species known to infect members of the *Papaver* genus.

Conidium dimensions were recorded from slides of 9 of 11 collections obtained. Conidia were measured at 400x magnification with the aid of a calibrated graticule. The first 100 conidia observed for each sample were measured, with misshapen, or

dehydrated, conidia ignored. For the remaining two isolates, numbers of conidia collected on slides were insufficient for counting. Statistical analysis of conidium dimensions was done using analysis of variance (ANOVA) with the software program Genstat (version 5; Lawes Agricultural Trust, Rothamsted, UK). Conidium length and width were analysed separately for significant differences between isolates, with isolates groupings based on least significant differences (LSDs) at the 95 % confidence level.

3.2.3. DNA amplification

ITS amplifications of all downy mildew collections obtained in this study (Table 3.1) were carried out using previously described universal primers ITS1 and ITS4, which target conserved regions in the 18S and 28S rRNA genes (Table 3.2; White, *et al.* 1990). Reaction mix contained 0.1 mM dNTPs, 0.25 µM of each primer, 1x PCR Buffer II (Applied Biosystems, Foster City, CA, USA), 1.5 mM $MgCl_2$, 50 µg bovine serum albumin (BSA), 1 U of AmpliTaq® Polymerase (Applied Biosystems) and 1 µL of conidia suspension in a total volume of 50 µL. Amplifications were carried out in a GeneAmp PCR Systems 2400 thermocycler (Perkin Elmer, Norwalk, CT, USA) using an initial denaturation step of 94 °C for one minute followed by 30 cycles of denaturation for 40 s at 92 °C, annealing for two minutes at 50 °C and extension for three minutes at 72 °C. This was concluded with a final extension for 10 min at 72 °C (Lindqvist *et al.* 1998). Products were separated in 2 % agarose (ICN Biochemicals, Ohio, USA) gels in 1x TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) subjected to 100 V for 49 min, stained with ethidium bromide (0.5 $mg.L^{-1}$) and visualised under UV light.

Table 3.1 List of species and specimens included in taxonomic study.

Species	Collection[1]	Host	Origin[2]	Source Year	Accession no.[3]
Hyaloperonospora niessleana[4]		*Alliaria petiolate*	GenBank		AF465763
H. parasitica[4]		*Thlaspi arvense*	GenBank		AF465759
Perofascia lepidii[4]		*Lepidium densiflorum*	GenBank		AF465760
Peronospora spp.	BO011	*Papaver somniferum*	Bothwell, TAS	2001	AY225482
Peronospora spp.	BU001	*Pap. somniferum*	Burnie, TAS	2000	AY225476
Peronospora spp.	HT001	*Pap. somniferum*	Hollow Tree (near Bothwell), TAS	2000	AY225477
Peronospora spp.	LA001	*Pap. somniferum*	Latrobe, TAS	2000	AY225478
Peronospora spp.	LO011	*Pap. somniferum*	Longford, TAS	2001	AY225472
Peronospora spp.	SA011	*Pap. somniferum*	Sasafrass, TAS	2001	AY225480
Peronospora spp.	SA012	*Pap. somniferum*	Sasafrass, TAS	2001	AY225474
Peronospora spp.	SD011	*Pap. somniferum*	Scottsdale, TAS	2001	AY225473
Peronospora spp.	SD012	*Pap. somniferum*	Scottsdale, TAS	2001	AY225479
Peronospora spp.	SO011	*Pap. somniferum*	Richmond, TAS	2001	AY225481
Peronospora spp.	WG001	*Pap. somniferum*	West Gawler (near Ulverstone), TAS	2001	AY225475
P. arborescens		*Pap. rhoeas*	GenBank		AF465761
P. cristata		*Meconopsis cambrica*	GenBank		AY374984

Table 3.1 con't.

Species	Collection[1]	Host	Origin[2]	Source Year	Accession no.[3]
P. destructor	TAS2	*Allium cepa*	TAS[5]	2001	AY225469
P. destructor		Unknown	GenBank		AB021712
P. farinose		*Chenopodium album*	GenBank		AF465762
P. manshurica		Unknown	GenBank		AB021711
P. rumicis		*Rumex acetosa*	GenBank		AF465758
P. sparsa	TAS1	*Rosa* sp.	TAS[5]	2001	AY225470
P. sparsa		*Rosa* sp.	GenBank		AF266783
P. viciae	SA014	*Pisum sativa*	Sasafrass, TAS	2001	AY225471
Phytophthora infestans		*Solanum tuberosum*	GenBank		AF266779

[1]Collection numbers for specimens sequenced in this study

[2]TAS = Tasmania, Australia

[3]GenBank accession no.

[4]Putative species names as proposed by Constantinescu & Fatehi (2002)

[5]Samples provided by Department of Primary Industries, Water and Environment, Tasmania, Australia

Conidia suspensions known to contain contaminants from non-oomycete species were subjected to an additional initial step in a semi-nested protocol using the primer DC6 (Table 3.2). DC6, used in conjunction with the primer ITS4, specifically amplifies members of the orders Pythiales and Peronosporales (Bonants *et al.* 1997). The first step of the nested protocol was carried out using the primers DC6 and ITS4 in a 25 µL reaction mixture. Reaction mixture contents and thermocycler settings were as described above. Following amplification, a 1 µL aliquot of the reaction product was substituted for the conidium suspension in the original reaction.

Table 3.2 PCR primers used in this study, their sequences and location within genomic ribosomal RNA gene repeat.

Primer	Sense	Sequence (5' → 3')	Location
DC6[1]	Forward	GAGGGACTTTTGGGTAATCA	18S gene
ITS1[2]	Forward	TCCGTAGGTGAACCTGCGG	18S gene
ITS4[2]	Reverse	TCCTCCGCTTATTGATATGC	28S gene

[1](Bonants *et al.* 1997)

[2](White *et al.* 1990)

3.2.4. DNA sequencing and phylogenetic analysis

Amplicons were purified using a QIAQuick™ PCR Purification Kit (QIAGEN, Hilden, Germany) according to manufacturer's specifications, and quantified by visualization under UV light following separation with a molecular mass standard (BioRad, Hercules, CA, USA) in 2 % agarose (ICN BioChemicals) gels in 1x TAE, subjected to 100 V for 49 min and stained with ethidium bromide (0.5 $mg.L^{-1}$). Amplicons were sequenced directly in both sense and antisense directions using an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems) and the primers ITS1 and ITS4 (3.2 pmol each), and sequences read

using an ABI 377 Automatic Sequencer (PE Applied Biosystems). All collections were sequenced twice, and a consensus sequence created from the duplicates. DNA sequences have been deposited in GenBank (Table 3.1).

DNA sequences of related species were obtained from GenBank (Table 3.1) and included in phylogenetic analyses. Sequence alignments were undertaken using Clustal W (Thompson *et al.* 1994). The sequence homology among individual sequences was determined by dividing the number of identical bases by the total number of bases. Phylogenetic distance and maximum parsimony analyses were conducted using software from the PHYLIP package of programs (Felsenstein 1989). Distance analysis was conducted using the neighbor-joining method with the program NEIGHBOR, based on Kimura 2-parameter distance matrices produced with the program DNADIST. Maximum parsimony analysis was conducted using the program DNAPARS, with gaps input into sequences to improve alignments treated as a fifth character state. All trees were drawn using the software TREEVIEW (Page 1996). The topology of all trees was supported by constructing 1000 bootstrap replicates with the program SEQBOOT, analysing as above and finding the maximum-rule and strict consensus tree using the program CONSENSE. The ITS sequence of *Phytophthora infestans* (GenBank accession no. AF266779) was added to all analyses and used as the root of all subsequent trees.

3.2.5. Primer design

Primers specific to sequences of Tasmanian collections of downy mildew were developed through visual comparison of the alignment of all sequences used in phylogenetic analysis. The specificity of potential primer regions was further tested by BLAST (Altschul *et al.* 1997) searching the GenBank database for compatible sequences. Following the identification of suitable target regions in the Tasmanian poppy downy mildew genome, primers were tested in PCR reactions against all Tasmanian downy mildew collections obtained. In addition, healthy poppy DNA, extracted using a phenol-choloroform protocol (see Chapter 6), was used as a negative control in all PCR reaction sets. The amplification mix consisted of 0.1 mM dNTPs, 0.25 μM of each primer, 1x PCR Buffer II (Applied Biosystems), 1.5 mM $MgCl_2$, 1 $\mu g.\mu L^{-1}$ BSA, 1 U of AmpliTaq® Polymerase (Applied Biosystems) and 1 μL of spore suspension, made up to 50 μL with sterile distilled water. Amplifications were carried out in a GeneAmp PCR Systems 2400 thermocycler (Perkin Elmer) using an initial denaturation at 94 °C for five minutes followed by 30 cycles of denaturation for 40 s at 92 °C, annealing for two minutes at 53 °C and extension for three minutes at 72 °C. This was concluded with a final extension for 10 min at 72 °C. Amplicons were separated in 2 % agarose gels in 1x TAE at 100 V for 49 min, stained with ethidium bromide (0.5 $mg.L^{-1}$) and visualised under UV light.

3.3. Results

3.3.1. Morphological observations

All downy mildew collections obtained from Tasmanian poppy crops displayed reproductive organs with general morphological characteristics consistent with *Peronospora* spp. known to infect *Papaver* spp.

Mean conidium lengths of downy mildew collections obtained from Tasmania varied between 24.3 and 26.4 µm, with the shortest and longest conidia recorded for collection BO001, and collections SA012, SD011 and BU001 respectively (Table 3.3). Mean conidium widths varied between 16.9 (collections HT001 and SA011) and 19.1 µm (collection SA012; Table 3.3).

Previously published mean conidium lengths for *P. arborescens* varied between 16.5 and 23.3 µm, while those of *P. cristata* varied between 21.0 and 24.7 µm (Table 3.3). Published mean *P. arborescens* conidium widths varied between 15.1 and 19.0 µm, while mean *P. cristata* conidium widths on *Papaver* spp. varied between 18.4 and 20.8 µm.

Table 3.3 Conidium dimensions of downy mildew species recorded on *Papaver* spp.

	Length (µm)			Width (µm)			
Specimen	**min.**	**mean**	**max.**	**min.**	**mean**	**max.**	**Host**
SA012 (2)[1]	20.0	26.4 a[2]	35.0	15.0	19.1 a	22.5	*Papaver somniferum*
SD011 (2)	20.0	26.4 a	34.0	12.5	17.4 c	24.0	*Pap. somniferum*
BU001 (2)	20.5	26.4 a	31.5	15.0	18.5 b	22.5	*Pap. somniferum*
SD012 (1)	21.5	25.7 b	30.0	15.0	18.4 b	22.5	*Pap. somniferum*
SA011 (1)	22.5	25.5 b	32.5	15.0	16.9 d	20.0	*Pap. somniferum*
HT001 (1)	20.5	25.3bc	35.0	14.0	16.9 d	20.0	*Pap. somniferum*
LO011 (1)	20.0	25.1bc	32.5	15.0	17.8 c	22.5	*Pap. somniferum*
SO011 (4)	20.0	24.8cd	29.0	15.0	17.8 c	21.5	*Pap. somniferum*
BO001 (3)	19.5	24.3 d	30.0	15.0	18.6 b	23.0	*Pap. somniferum*
Peronospora arborescens[3]		16.5			15.1		*Pap. rhoeas*
P. arborescens[4]	13.0	18.7 – 23.3	29.0	11.0	16.3	21.0	*Pap. somniferum*
P. arborescens[5]	19.8	22.8	24.2	18.0	19.0	19.4	*Pap. somniferum*
P. arborescens[6]		18.0 -20.6			15.8 -16.9		*Pap. dubium*
P. arborescens[7]	18.4	23.1	30.0	15.2	19.0	26.4	*Pap. somniferum*
P. arborescens[8]	17.0	19.4	22.0	15.0	16.3	17.5	*Pap. somniferum*
P. arborescens[9]	18.0		26.0	16.0		20.0	Unknown
P. cristata[3]		21.0			18.6		*Pap. argemone*
P. cristata[6]		22.7 -24.7			18.4 -20.8		*Pap. argemone*

[1]Numbers in parentheses indicate the group number of the collection based on ITS sequence

[2]Letters indicate significant ($P < 0.05$) differences between; length LSD = 0.681; width LSD = 0.466

[3](Gaumann 1923) [5](Behr 1956) [7](Kothari & Prasad 1970) [9](Francis 1981)

[4](Yossifovitch 1929) [6](Gustavsson 1959) [8](Scharif 1970)

3.3.2. Sequence analysis

The 11 collections of downy mildew pathogens obtained from *Pap. somniferum* in Tasmania were differentiated into four ITS sequence types. These sequences differed at a total of four nucleotide positions in the 5.8S and ITS2 regions (Fig. 3.1). Groups 1 to 4 consisted of five, four, and two single collections, respectively. Groups 2, 3 and 4 differed from Group 1 by two nucleotides in the 5.8S gene, one and two nucleotides in the ITS2, respectively. An apparent relationship existed between ITS grouping and conidium length. No difference ($P > 0.05$) in conidium length was observed between collections within the individual DNA sequence Groups 1 to 4. The conidia of all Group 2 collections were longer ($P < 0.05$) than those of all other collections (Table 3). Group 1 collections had longer ($P < 0.05$) conidia than Group 3 collections. No difference ($P > 0.05$) was observed in mean conidium length between Group 3 and Group 4 collections (Table 3.3).

The sequence homology for the ITS region among samples collected from Tasmanian poppy crops and *P. arborescens* (GenBank accession no. AF465761; Constantinescu & Fatehi 2002) varied between 92.1 and 92.4 % (Table 3.4). Sequence homology among Tasmanian collections and *P. cristata* (GenBank accession no. AY374984) varied between 98.8 and 99.1 %. The Tasmanian collections of *P. destructor* and *P. sparsa* had sequence homologies of 98.8 and 97.0 % with the relevant sequences obtained from GenBank (accession nos. AB021712 and AF266783, respectively).

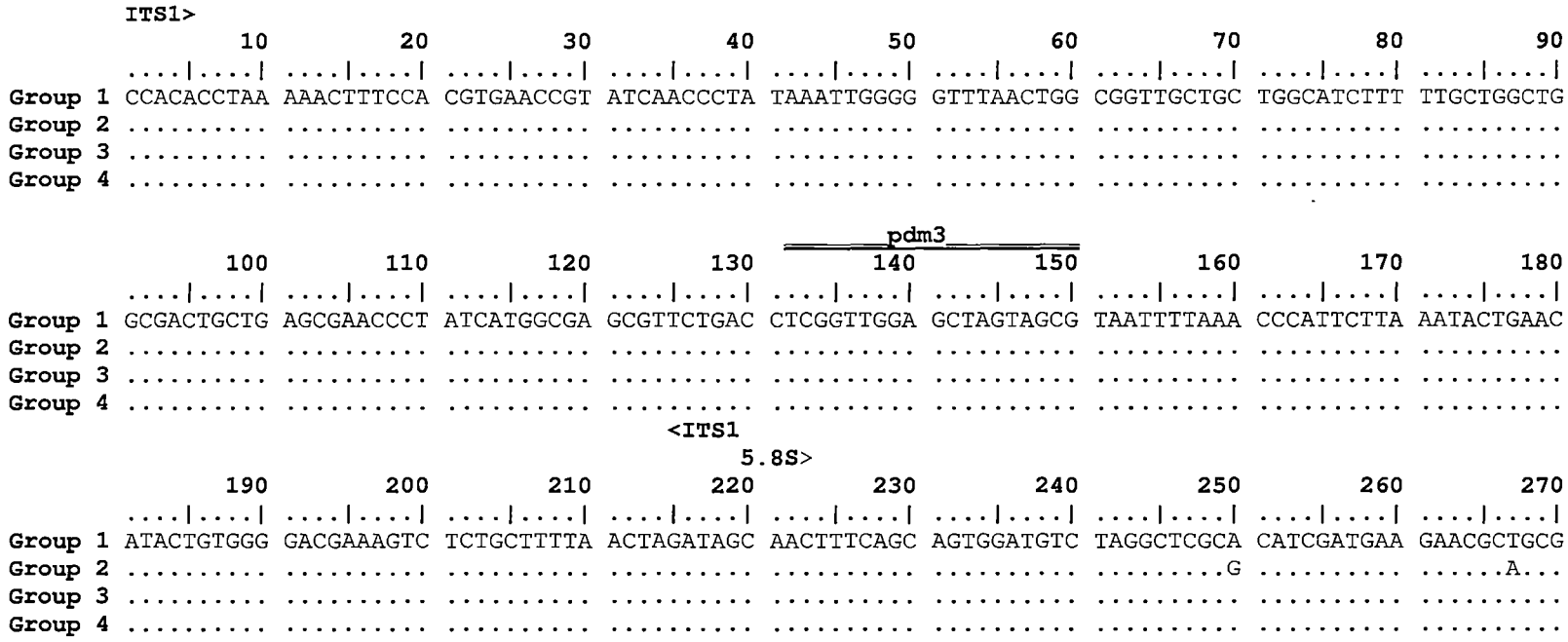


Fig. 3.1 Aligned DNA sequences of the complete ITS (ITS1, 5.8S and ITS2) region of rDNA of specimens of downy mildew collected from oilseed poppy in Tasmania. Group 1 includes Tasmanian poppy downy mildew collections LO011, HT001, LA001, SD012 and SA011. Group 2 includes collections BU001, SA012, WG001 and SD011. Group 3 includes collection BO001. Group 4 includes collection SO011. The start (>) and end (<) of the ITS1, 5.8S and ITS2 regions are indicated, as are the positions of the primers pdm3 and pdm4.

Fig. 3.1 cont.

```
                280        290        300        310        320        330        340        350        360
          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
Group 1 AACTGCGATA CGTAATGCGA ATTGCAGGAT TCAGTGAGTC ATCGAAATTT TGAACGCATA TTGCACTTCC GGGTTATCCC TGGGAGTATG
Group 2 .......... .......... .......... .......... .......... .......... .......... .......... ..........
Group 3 .......... .......... .......... .......... .......... .......... .......... .......... ..........
Group 4 .......... .......... .......... .......... .......... .......... .......... .......... ..........
                     <5.8S
                          ITS2>
                370        380        390        400        410        420        430        440        450
          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
Group 1 CCTGTATCAG TGTCCGTACA TCAAACTTGG TTTTCTTCTT TCCGTGTAGT CGGTGGAGGA TATGCCAGAT GTGAAGTGTC TTGCGGCTGG
Group 2 .......... .......... .......... .......... .......... .......... .......... .......... ..........
Group 3 .......... .......... .......... .......... .......... .......... .......... .......... ..........
Group 4 .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                                                        pdm4
                460        470        480        490        500        510        520        530        540
          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
Group 1 TTTTCGGATC GGCTGTGAGT CCTTTGAAAT GTACAGAACT GACTCTCTTT GTTTGAAAAG CGTGGCGTTG CTGGTTGTGG AGGCTGTCAG
Group 2 .......... .......... .......... .......... .......... .......... .......... .......... ..........
Group 3 .......... .......... .......... .......... .......... .......... .......... .......... ..........
Group 4 .......... .......... .......... .......... .......... .......... .......... .......... ........G.

                550        560        570        580        590        600        610        620        630
          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
Group 1 TATGGCTAGT CGGCGACCGG TTTGTCTGCT ATGGCATCAT GGAGGAGTGT TCGATTCGCG GTATGGTTGG CTTCGGCTAA ACAGGCGCTT
Group 2 .......... .......... .......... .......... .......... .......... .......... .......... ..........
Group 3 .......... .......... .......... .......... .......... .......... .......... .......... ..........
Group 4 .......... .......... .......... .......... .......... .......... .......... .......... ..........
```

Fig. 3.1 cont.

```
                640        650        660        670        680        690        700        710        720
          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
Group 1 ATTGGACGTT CTTTCTGCTA TGGCGGTATG AACTGGTGAA CCGTAGTTCA TGCATGACTT GGCTTTTGAA TCGGCTTTGC TGTGCGAAGT
Group 2 .......... .......... .......... .......... .......... .......... .......... .......... ..........
Group 3 .......... .......... .......... .......... .......... .......... .......... .......... ..........
Group 4 .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                                                                                  <ITS2
                730        740        750        760        770        780        790
          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|...
Group 1 AGAGTGACAG TTTCGGCTGT CGAGGGTCGA CCCATTTGGG AACTTGTGCT GTGCGACTTC GGTTGCGTGG CATCTCAA
Group 2 .......... .......... .......... .......... .......... .......... .......... ........
Group 3 .......... .......... .......... .......... .......T.. .......... .......... ........
Group 4 .......... .......... .......... .......... .......T.. .......... .......... ........
```

Table 3.4 Sequence homologies of *Peronospora* spp., including poppy downy mildew collections, based on the ITS region of rDNA.

Sequence	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 Group 1[1]	1.000	0.997	0.998	0.997	0.991	0.924	0.952	0.960	0.933	0.911	0.942	0.913	0.908	0.903
2 Group 2[1]	–	1.000	0.996	0.994	0.988	0.921	0.955	0.957	0.931	0.909	0.940	0.910	0.905	0.905
3 Group 3[1]	–	–	1.000	0.998	0.989	0.922	0.951	0.958	0.932	0.910	0.941	0.912	0.907	0.901
4 Group 4[1]	–	–	–	1.000	0.988	0.922	0.950	0.957	0.932	0.910	0.940	0.912	0.907	0.901
5 *P. cristate*	–	–	–	–	1.000	0.916	0.943	0.951	0.926	0.906	0.933	0.905	0.900	0.895
6 *P. arborescens*	–	–	–	–	–	1.000	0.919	0.925	0.905	0.918	0.915	0.899	0.911	0.906
7 *P. destructor* TAS2	–	–	–	–	–	–	1.000	0.988	0.923	0.904	0.930	0.903	0.903	0.904
8 *P. destructor*	–	–	–	–	–	–	–	1.000	0.930	0.906	0.935	0.909	0.909	0.905
9 *P. farinosa*	–	–	–	–	–	–	–	–	1.000	0.894	0.924	0.897	0.893	0.888
10 *P. manshurica*	–	–	–	–	–	–	–	–	–	1.000	0.900	0.903	0.911	0.916
11 *P. rumicis*	–	–	–	–	–	–	–	–	–	–	1.000	0.902	0.898	0.890
12 *P. sparsa* TAS1	–	–	–	–	–	–	–	–	–	–	–	1.000	0.970	0.892
13 *P. sparsa*	–	–	–	–	–	–	–	–	–	–	–	–	1.000	0.900
14 *P. viciae* SA014	–	–	–	–	–	–	–	–	–	–	–	–	–	1.000

[1]Group 1 includes Tasmanian poppy downy mildew collections LO011, HT001, LA001, SD012 and SA011. Group 2 includes collections BU001, SA012, WG001 and SD011. Group 3 includes collection BO001. Group 4 includes collection SO011.

3.3.3. Phylogenetic analysis

The topologies of phylogenetic trees produced by distance and maximum parsimony methods were almost identical. The topology of the single most parsimonious tree (Fig. 3.2), selected from twelve equally parsimonious trees by majority rule and strict consensus analysis, varied from that of the tree produced by distance based methods (Fig. 3.3) at only two points. The arrangement of the clade containing Tasmanian poppy downy mildew collections and *P. cristata* varied between methods, while *P. farinosa* and *P. ruminicis* were placed into a single clade by parsimony analysis. *Peronospora cristata* was grouped with Tasmanian poppy downy mildew collections with bootstrapping values of 93 % (Fig. 3.3) and 74 % (Fig. 3.2) for distance and parsimony analysis, respectively. Both analyses indicated that *P. arborescens* was more distantly related to this clade than *P. destructor*, *P. rumicis* and *P. farinosa*.

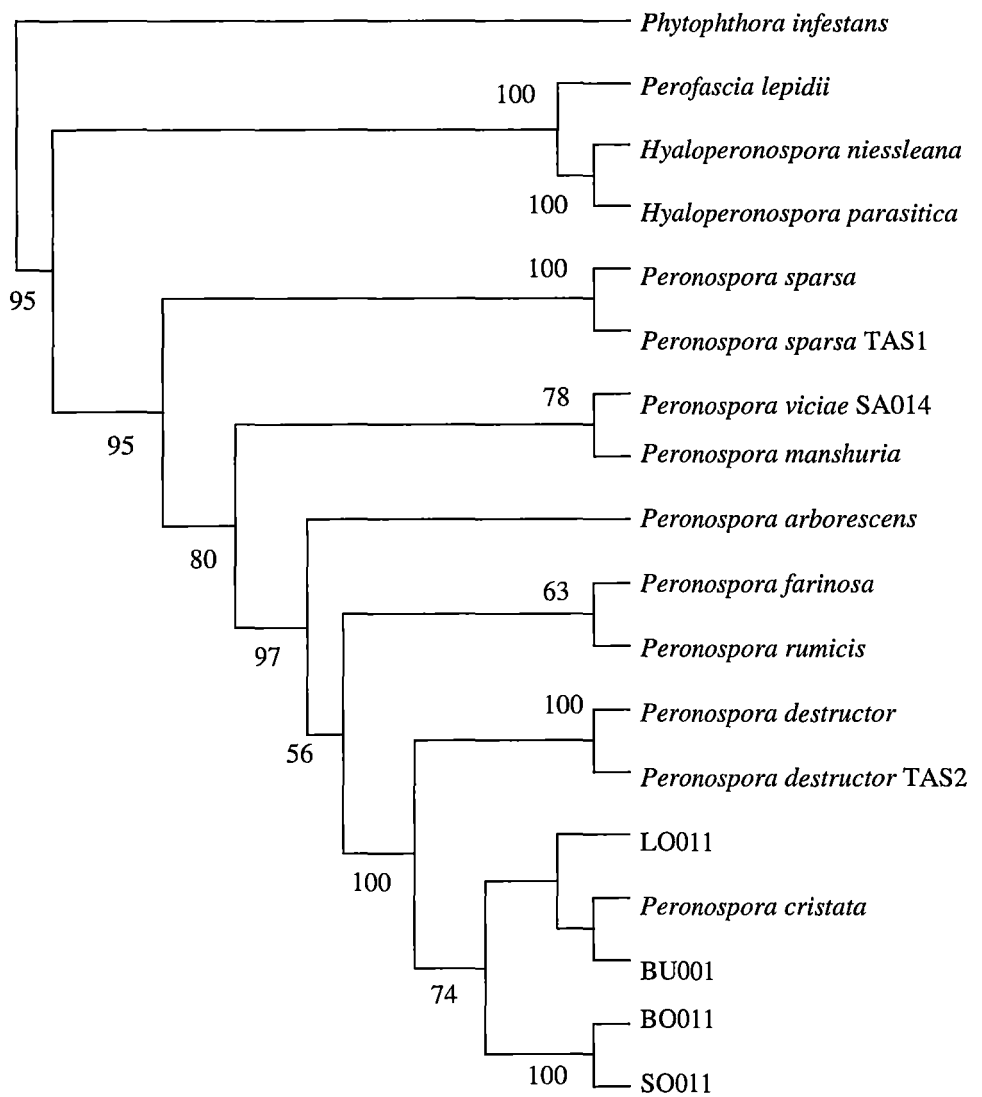


Fig. 3.2 Phylogenetic tree indicating the relationships among downy mildew species based on DNA sequences of the ITS1, 5.8S and ITS2 region of rDNA. Collections LO011, BU001, BO001 and SO011 represent Tasmanian poppy downy mildew collections from Groups 1, 2, 3 and 4 respectively. Cladogram created using maximum parsimony method (using DNAPARS program). Majority-rule and strict consensus tree selected from 12 equally parsimonious trees using CONSENSE program. Values in branches show bootstrap percentages from 1000 replicates (only values > 50 % shown). *Phytophthora infestans* was selected as outgroup.

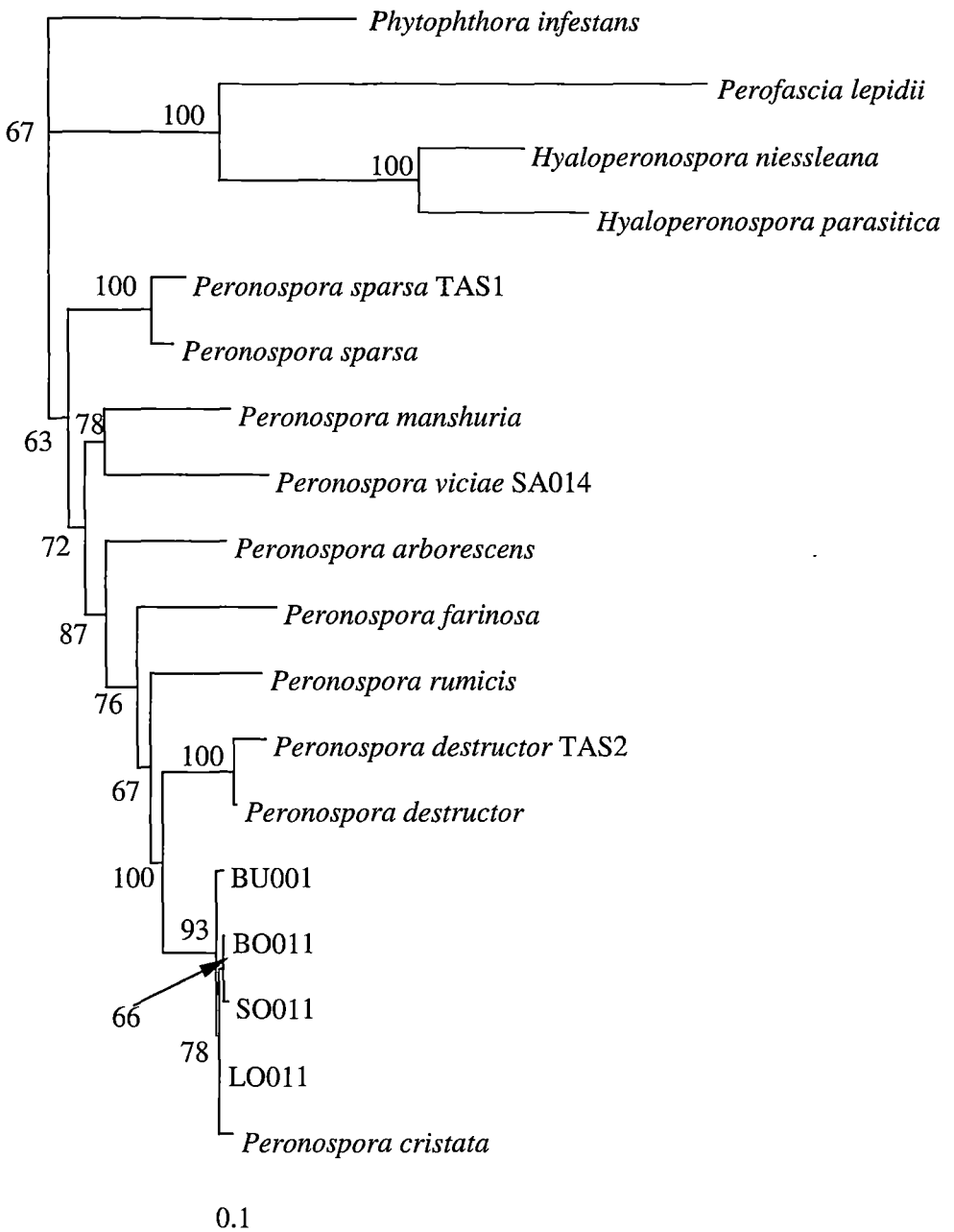


Fig. 3.3 Phylogenetic tree indicating the relationships among downy mildew species based on DNA sequences of the ITS2, 5.8S and ITS2 region of rDNA. Collections LO011, BU001, BO001 and SO011 represent Tasmanian poppy downy mildew collections from Groups 1, 2, 3 and 4 respectively. Phylogram created using neighbor-joining method (using NEIGHBOR program) based on genetic distances calculated by the Kimura 2-parameter model (using DNADIST program). Scale bar indicates 0.1 expected nucleotide substitutions per site. Values in branches show bootstrap percentages from 1000 replicates (only values > 50 % shown). *Phytophthora infestans* was selected as outgroup.

3.3.4. Primer Design

The primer pair pdm3/pdm4 (Table 3.5) produced a single DNA band approximately 389 base pairs (bp) in size for all Tasmanian poppy downy mildew specimens collected in this study. No bands were produced when tested against specimens of *P. destructor*, *P. viciae* or *P. sparsa* (Fig. 3.4). No bands were produced by samples containing healthy poppy DNA as a negative control.

Table 3.5 PCR primers developed in this study for the specific detection of Tasmanian poppy downy mildew collections, their sequences and location within genomic ribosomal RNA gene repeat.

Primer	Sense	Sequence (5' → 3')	Location
pdm3	Forward	TCGGTTGGAGCTAGTAGCG	ITS1
pdm4	Reverse	CAACGCCACGCTTTTCA	ITS2

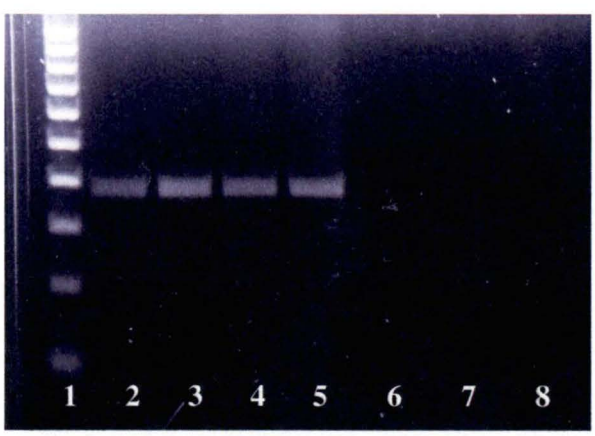


Fig. 3.4 Reaction of downy mildew collections to the PCR primer pair pdm3/pdm4. Lane 1: 100 bp increment ruler with lowermost band equal to 100 bp; lanes 2-5: Tasmanian poppy downy mildew collections BU001, LO011, SO011, BO001 respectively; lane 6: *Peronospora destructor* TAS2; lane 7: *P. sparsa* TAS1; lane 8: *P. viciae* SA014.

3.4. Discussion

Mean conidium dimensions of specimens of downy mildew pathogens collected from Tasmanian poppy crops were generally longer, but not wider, than those in previously published accounts for *Peronospora* spp. known to infect *Papaver* spp. Based on published records, *P. arborescens* conidium dimensions can range between 13.0 to 30.0 µm by 8.5 to 26.4 µm, whilst *P. cristata* range between 14.0 to 31.6 µm by 14.2 to 25.6 µm. In the present study, conidium dimensions varied between 19.5 to 35.0 µm by 12.5 to 24.0 µm. This overlap highlights the difficulty of differentiating *Peronospora* spp. based only on spore dimensions, and the need to find alternative means of identification. It is probable that some of the overlap in conidium dimensions between *P. cristata* and *P. arborescens* can be attributed to misclassification of species at the time. To clearly define the morphological dimensions of these two species it is first necessary to correctly identify collections, using methods such as the molecular techniques used in the present study.

Based on ITS sequences, Tasmanian poppy downy mildew collections were divided into four distinct groups, based on variations at a few nucleotide sites over the ITS region. This level of variation is comparable to that recorded from collections of *P. sparsa* on arctic bramble in a similar study in Finland, where variations were recorded at a total of six nucleotide sites over the ITS1 and ITS2 regions (Lindqvist *et al.* 1998). In the present study, no geographic basis for the groupings based on ITS sequences was observed, which may be indicative of a more recent introduction for the pathogen, primary spread by seed, and/or long distance dispersal from a single geographic region each season. To date, no studies have identified the principal source of primary inoculum for Tasmanian epidemics. However, there was

a general association between conidium length and ITS sequence. All Group 2 collections had longer conidia than collections from other groups. Group 3 and Group 4 collections had the shortest conidia, although conidia from collection SO011 in Group 4 were not shorter that some members of Group 1. There was no statistically significant difference in length among collections within groups. This relationship between ITS sequence and conidium morphology was not apparent in recorded conidium widths. Due to the small number of collections used in the present study, additional work is required to confirm any relationship between ITS sequence and conidium length.

Sequence analysis of the ITS sequence of Tasmanian poppy downy mildew collections indicated that they are the same species as that identified as *P. cristata* (GenBank accession no. AY374984). Tasmanian poppy downy mildew collections shared 99 % sequence homology with *P. cristata*, which was comparable to the sequence homology observed between a Tasmanian collection of *P. destructor* and its respective sequence obtained from GenBank (99 %), and greater than that observed for *P. sparsa* (97 %). Sequence homology among Tasmanian poppy downy mildew collections and the ITS sequence identified as *P. arborescens* (GenBank accession no. AF465761) was only 92 %.

Phylogenetic analyses of the ITS region consistently indicated that Tasmanian poppy downy mildew collections shared a common ancestor with all *Peronospora* sequences used in the present study. Analysis of the 28S rDNA (LSU) of members of the Peronosporomycetes indicated that the genus *Peronospora* is a polyphyletic group (Riethmuller *et al.* 2002). In addition, Riethmuller *et al.* (2002) observed that most *Peronospora* spp. infecting Fabaceae hosts and all *Peronospora* spp. infecting

Ranunculaceae hosts, used in their study belonged to single clades. A monophyletic clade consisting of all *Peronospora* spp. infecting Brassicaceae hosts was more distantly related to the majority of *Peronospora* spp. (Riethmuller *et al.* 2002). Analysis of the ITS rDNA of *Peronospora* spp. led to the creation of the genera *Hyaloperonospora* and *Perofascia* by Constantinescu and Fatehi (2002), from *Peronospora* spp. infecting Brassicaceae hosts, which was supported by their more distant relationship with the genus *Peronospora* in the present study. However, the apparent relationship between pathogen and host systematics was not fully supported by the results of the present study. Tasmanian poppy downy mildew collections were most closely related to *P. cristata* by both distance and maximum parsimony methods and more closely related to *P. destructor*, *P. rumincis* and *P. farinosa* than to *Peronospora arborescens*.

Thus, molecular evidence indicates that two species, *P. arborescens* and *P. cristata*, are able to infect *Papaver* spp., while the Tasmanian poppy downy mildew pathogen appears to be *P. cristata*. The GenBank sequences of *P. arborescens* and *P. cristata* used in the present study were obtained from pathogens collected from *Pap. rhoeas* and *M. cambrica*, respectively. *Papaver rhoeas* is the host from which *P. arborescens* was first recorded (Berkeley 1846), while *M. cambrica* is recorded as a host of *P. cristata*, with *P. arborescens* restricted to cultivated members of *Meconopsis* spp. (Reid 1969). Therefore, it is probable that the two sequences in question were correctly classified. The geographic origins of the *P. cristata* and *P. arborescens* sequences, England and Romania, respectively, also indicate that the Tasmanian downy mildew pathogen's sequence would be closest to *P. cristata*. The Tasmanian poppy industry was founded from England, while to the author's

knowledge little movement of crop material has taken place between Romania and Tasmania.

Hall (1996) argued that while the morphology of asexual structures can be used to reliably differentiate species of downy mildew pathogens to the genus level, molecular techniques such as those incorporating the sequencing of rDNA may need to be used to differentiate at the species level. To date, examples of the use of ITS sequences to redefine the taxonomy of *Peronospora* species include *P. rubi* and *P. sparsa*, which have been shown to conspecific (Kokko *et al.* 1999), and *Peronospora* spp. parasitic on Brassicaceae, which have been redefined into two new genera (Constantinescu & Fatehi 2002).

In addition to the identification of the causal organism of downy mildew in Tasmanian poppy crops, this study has also led to the development of PCR primers for the specific detection of this pathogen. DNA sequence comparison indicated that the primers would differentiate between the species *P. cristata* and *P. arborescens*. BLAST searches of the GenBank database, which is known to include sequences from the common poppy pathogens *Pleospora papaveracea*, *Dendryphrion penicillatum* and *Entyloma fuscum*, and testing against the most common *Peronospora* species in Tasmania did not indicate any potential cross reactions. These results indicate that these primers specifically detect *P. cristata*. Future studies will incorporate these primers into the assessment of the presence of downy mildew inoculum in seed.

Evidence from this study suggests that two distinct *Peronospora* species are able to infect *Papaver* spp., and that the downy mildew pathogen in Tasmanian poppy crops

is *P. cristata*, not *P. arborescens*. To our knowledge, this constitutes the first record of *P. cristata* infecting *Pap. somniferum*. Further work is required to determine whether *P. arborescens*, as recorded by Constantinescu and Fatehi (2002) is also able to infect *Pap. somniferum*. If this is not the case then it appears that previous studies attributing downy mildew of opium poppy to *P. arborescens* should be reappraised.

4. Epidemic development of *Peronospora cristata* in oilseed poppy

4.1. Introduction

Spatiotemporal analysis of epidemics can be used to provide information on the dynamics of epidemic development, including the importance of primary inoculum and the mechanisms of pathogen dispersal. Knowledge of these characteristics can then be used in the development of improved control strategies.

Temporal analysis of epidemics is typically done through the fitting of standard disease progress models, or curves (DPC), to collected data (Madden 1980). Quantification of disease progression allows for the evaluation of control strategies, prediction of future levels of disease and verification of disease simulators and forecasters (Madden 1980). The type of DPC can also be indicative of the structure of epidemic development. van der Plank (1963) divided disease epidemics into simple interest, or monocyclic, and compound interest, or polycyclic, diseases. Monocyclic diseases are only able to complete a single disease cycle within a given season, and as such epidemics of this type are driven by a primary inoculum source. Polycyclic disease epidemics are able to complete two or more disease cycles within a single season, and therefore, in addition to the initial primary inoculum, develop secondary inoculum from within the crop following primary infections. Monocyclic disease epidemics are typified by the monomolecular disease curve, while polycyclic diseases can be described by the exponential, logistic and Gompertz disease curves (van der Plank, 1963). In practice, the type of DPC cannot be used as direct evidence of a monocyclic or polycyclic disease, however the DPC can be used to indicate whether a primary or secondary inoculum is the predominant source of infection for epidemic development in the field.

Spatial analysis techniques can be divided into two broad categories; point-pattern and correlation analyses (Upton & Fingleton 1985). When conducting spatial analyses it is best to use methodologies from both categories to provide a fuller interpretation of the spatial pattern within a field. Distribution fitting, a point-pattern type analysis, is used to measure the heterogeneity of counts in sampling units, independent of the spatial structure of those sampling units (Hughes & Madden 1993). Median runs analysis, a correlation type analysis, assesses the relationship between the individual disease scores of spatially referenced sampling units, while not directly using the disease incidence or disease severity measurements (Gibbons 1985). Spatial analysis by distance indices (SADIE) a second correlation type analysis, differs as it uses data in the form of point-patterns and is therefore conditioned upon the inherent heterogeneity within a data set (Perry 1995).

Spatiotemporal analysis provides a means of combining temporal and spatial data into a single analysis. SADIE has recently been extended to provide a new method of spatiotemporal analysis (Winder *et al.* 2001). This uses the SADIE method of spatial association to assess the relationship between spatial patterns within a field at differing time periods.

The relationship between the spatial patterns of two different factors in the same spatial area can be assessed using spatial association analyses. An extension of SADIE provides one means of measuring spatial association (Winder *et al.* 2001).

The objectives of this work were to (1) determine the characteristic disease progress curve of poppy downy mildew, (2) quantify the spatial pattern of disease at different

time periods in the crop development, (3) assess the spatiotemporal association between these time periods, and (4) assess the relationship between crop characteristics and disease development. It was hoped that analysis of these characteristics would provide evidence as to the driving force behind the development of epidemics, potentially allowing for the implementation targeted control strategies towards either primary or secondary inoculum. In addition, information on the relationship between crop growth characteristics and disease development may allow for modification to the crop growth habit for improved diseased control. Identification of the key time periods for disease development may allow for improved targeting of control strategies for the disease.[1]

[1] With the exception of analyses of the relationship between crop characteristics and disease development, the work in this chapter has been previously published by Scott, J. B., Hay, F. S., Wilson, C. R., Cotterill, P. J. & Fist, A. J. (2003). Spatiotemporal analysis of epiphytotics of downy milew of oilseed poppy in Tasmania, Australia. *Phytopathology* **93**: 752-757.

4.2. Materials and methods

4.2.1. Field trials

Field trials were established at the TAFE/Freer Farm, Burnie (S41° 04', E145° 51'; Appendix I) in the 2000/2001 (2000) and 2001/2002 (2001) growing seasons. Field trials were sown on the 26th and 14th September in 2000 and 2001, respectively. Sowing rate, fertiliser application and weed control were all based on commercial recomendations (Table 4.1). The 2000 trial consisted of 50 plots arranged in a 5 by 10 lattice, each 8 by 12 m, in a commercial field that received a single eradicative fungicide application 67 days after sowing (DAS; Table 4.1). The 2001 trial consisted of 64 plots arranged in a 4 by 16 lattice, each 5 by 5 m, which were not treated with fungicides. A single leaf was sampled from the lowermost five nodes of 30 arbitrarily selected plants from each plot, at six times during each season. A preliminary study (Appendix III) was carried out to determine the effect of leaf age on infection. Samples were collected at 53, 63, 73, 83, 93 and 103 DAS in 2000, and 45, 55, 65, 75, 85 and 95 DAS in 2001. Leaves were scored for severity of symptoms, using percentage leaf area diagrams prepared by the author using the methodology of Falloon *et al.* (1995; Appendix IV). Leaves were incubated in sealed plastic containers over a wet tissue in the dark at 12 °C and 100 % relative humidity for at least 12 hours and no longer than 72 hours. Downy mildew infection was confirmed by microscopic observation of sporulation (65x magnification).

Prior to initial sampling in each season, the plant density of individual plots was determined. A hoop, 78 cm in diameter (area = 0.48 m^2), was randomly placed within each plot and poppy plants within the hoop counted. Counts were replicated 3 times for each plot. Average plant counts were converted to plants.m^{-2}.

Four days prior to commercial harvest (Table 4.1), 10 m^2 areas from within each quadrat were hand harvested. From these samples, the capsule dry weight and alkaloid content for each quadrat was determined. Capsule weight and alkaloid contents were determined using the standard practice of Tasmanian Alkaloids Pty. Ltd. Harvested capsules were air dried and weighed. Samples were ground to a particle size of less than 2 mm in diameter, and 3.5 g of sample added to 0.3 g of lime and 50 mL of distilled water and shaken for 30 min. Oxalic acid (12.5 mL) was then added to the mix and filtered. Alkaloid analysis was then conducted using a Waters high pressure liquid chromatogram (HPLC; Waters Corporation, Milford, Massachusetts, USA) with a Phenomenex Phenosphere 5 µm 5CX 80A column.

Table 4.1 Summary of agronomic activities for field trials at the TAFE/Freer Farm, Burnie, Tasmania, in 2000 and 2001 growing seasons.

Season	Activity	Chemical	Active constituent	Rate	Application (DAS[1])
2000	sowing			900 $g.ha^{-1}$	0
	fertilisers	Superphosphate	4:16:11, N:P:K	375 $kg.ha^{-1}$	0
		Nitram (Incitec Fertilizers Ltd., QLD, Australia)	ammonium nitrate (34:0:0, N:P:K)	250 $kg.ha^{-1}$	75
	herbicides	Command® 480 EC Herbicide (FMC® (Chemicals), Pty. Ltd., QLD, Australia)	clomazone 480 $g.L^{-1}$, hydrocarbon liquid 450 $g.L^{-1}$	500 $mL.ha^{-1}$	35
		Asulox® Selective Herbicide (Bayer Cropscience Pty. Ltd., VIC, Australia)	Asulam 400$g.L^{-1}$	5 $L.ha^{-1}$	35
		Reglone Non-Residual Herbicide (Syngenta Crop Protection Pty. Ltd, NSW, Australia).	diquat dibromide monohydrate 200 $g.L^{-1}$	1 $L.ha^{-1}$	43
	fungicides	Ridomil Gold MZ Systemic & Protective Fungicide (Syngenta Crop Protection Pty. Ltd, NSW, Australia).	mancozeb 640 $g.kg^{-1}$, metalaxyl 40 $g.kg^{-1}$	2.5 $kg.ha^{-1}$	67
	harvest				143

Table 4.1 cont.

Season	Activity	Chemical	Active constituent	Rate	Application (DAS[1])
2001	sowing			900 $g.ha^{-1}$	0
	fertilisers	Superphosphate	4:16:11, N:P:K	375 $kg.ha^{-1}$	0
	herbicides	Command® 480 EC Herbicide	clomazone 480 $g.L^{-1}$, hydrocarbon liquid 450 $g.L^{-1}$	500 $mL.ha^{-1}$	44
		Reglone Non-Residual Herbicide	diquat dibromide monohydrate 200 $g.L^{-1}$	800 $mL.ha^{-1}$	48
	harvest				167

[1]days after sowing

4.2.2. Temporal analysis

The disease progress within each trial was assessed by fitting the exponential, monomolecular, logistic, Gompertz and linear epidemic models to the data and selecting the model of best fit (Neher *et al.* 1997). The linear form of each model was used to assess goodness-of-fit through regression to find the co-efficient of determination (R^2), and visual comparison of the plots of the observed versus expected values. To determine the model of best fit, data were back-transformed, and the new R^2 values compared (Neher *et al.* 1997).

4.2.3. Spatial analysis

Spatial analyses of disease incidence and severity data at different times during each season were conducted using three separate analysis techniques; distribution fitting, median runs analysis and SADIE. Spatial analyses were conducted only when the overall disease incidence, or severity, ranged between 5 and 95 %.

Heterogeneity analysis

The beta-binomial and binomial distributions were fitted to the available incidence data for each season to assess the heterogeneity of each data set using the software program BBD (Madden & Hughes 1994). A good fit of a data set to the binomial distribution was suggestive of random disease distribution. A good fit of a data set to the beta-binomial distribution was suggestive of overdispersion, or spatial aggregation. The log-likelihood ratio statistic (*LRS*) was used to determine if beta-binomial distribution provided a better fit to the individual data sets than the

binomial distribution (Turechek & Madden 1999b). *LRS* was calculated using the formula:

$$LRS = 2 \times [\log(\text{likBBD}) - \log(\text{likBD})]$$

Equation 4.1

where log(likBBD) and log(likBD) are the log-likelihoods for the beta-binomial and binomial distributions respectively. The *LRS* was assumed to a have a χ^2 distribution with one degree of freedom, and a null hypothesis that there was no difference between the two distributions (Turechek & Madden 1999a). Rejection of the null hypothesis was evidence for the beta-binomial distribution (Madden & Hughes 1994). The index of dispersion, *D*, the ratio of the observed variance to the expected variance under the binomial distribution, was also calculated to provide a measure of the degree of aggregation (Madden & Hughes 1994).

Correlation analyses

Median runs analyses, an adaptation of ordinary runs analysis (Gibbons 1985), were conducted on the available incidence and severity data for each season. A run was defined as a series of one or more plots with the same disease status bordered by plots with a different disease status (Gibbons 1985). To adapt the data sets for median runs analysis, sampling units with values greater than the data set median were given the disease status 1 (Gibbons 1985). All other sampling units were assigned a 0. The individual rows and columns of each data set were combined into a single row and column respectively. Significance was tested using a standard *Z*-test statistic to compare the observed number of runs with the expected number of

runs under a null hypothesis of randomness. Observed runs significantly lower than expected runs suggested spatial aggregation (Gibbons 1985).

Spatial distribution of incidence and severity data for each season were also analysed using SADIE (Perry 1995; Perry *et al.* 1999). SADIE uses a transportation algorithm to rearrange spatially referenced data and determine the shortest distances individual count units need to travel to achieve both 'regular' and 'crowded' spatial patterns, whilst maintaining the overall number of individuals (Perry 1995). These distances are then summed for each pattern to give the overall 'distance to regularity' and 'distance to crowding'. Random reallocation of sampling unit values and recalculating distances to regularity and crowding was then used to find expected (mean) and percentile values based on frequency distribution of distances. Indices of aggregation were then calculated for both regularity (I_a) and crowding (J_a). I_a was equal to the ratio of the observed and expected distances to regularity. J_a was equal to the ratio of expected to observed distances to crowding. For both indices, values greater than 1 indicated spatial aggregation, less than 1 indicated regularity and equal to 1 indicated randomness (Perry 1995). A one-sided test for aggregation was used. For all analyses, the maximum 5967 randomizations were used. To accommodate the requirement within SADIE for discrete integer values, disease severity (%) data were transformed by multiplying by 10 and rounding to the nearest integer. The clustering indices were plotted in contour plots using SigmaPlot software (version 7.0; SPSS Inc., Chicago, USA) to map patch and gap clusters within fields. Clustering indices were a measure of the net distance needed to travel by individuals at each sampling unit to achieve regularity (Perry *et al.* 1999). Net movement of individuals out of a sampling unit occurs from those sampling units with counts greater than the field mean and were termed outflows (v_i), whilst net movement into

a sampling unit, occurring when counts are less than the field mean, were termed inflows (v_j) and were negative in value. Patch clusters, areas of significantly high individual counts, were defined as areas with v_i greater than the 95th percentile of v_i from the randomizations (Perry *et al.* 1999). Gap clusters, areas of significantly low individual counts, were defined as areas with v_j less than the 95th percentile of v_j from randomizations (Perry *et al.* 1999). When more than one patch cluster was present in a given data set J_a was ignored (Perry 1995).

4.2.4. Spatiotemporal analysis

Spatiotemporal analysis in SADIE uses the assumption that once spatial pattern has been established for the individual time periods, the relationship between pairs of time periods can be analysed by measuring their spatial association (Winder *et al.* 2001). Spatial association in SADIE first measured local association (χ_k) for a sampling unit by comparison of the clustering indices for each time period using the formula:

$$\chi_k = \frac{n(z_{k1} - q_1)(z_{k2} - q_2)}{\left[\sum_k (z_{k1} - q_1)^2 \sum_k (z_{k2} - q_2)^2\right]^{1/2}}$$

Equation 4.2

where z_{k1} and z_{k2} were the clustering indices at the k^{th} sampling unit for time periods one and two respectively, q_1 and q_2 were the mean indices for each time period and n was the total number of sampling units. The significance of X was then determined

through the random reallocation of z_k values amongst the sampling units, after correction for small-scale autocorrelation in z_k in either population (Dutilleul 1993). In this work, spatial-temporal association was assessed between all time periods assessed for spatial pattern and between incidence and severity data sets for each season. In all cases, the maximum 9999 randomisations were conducted. A two-tailed test was used with a null hypothesis of no association against the alternatives of spatiotemporal association, indicated by positive values of *X*, or spatiotemporal dissociation, indicated by negative values of *X* (Winder *et al.* 2001).

4.2.5. Spatial association

To assess overall spatial association, area under disease progress curves (*AUDPC*) were calculated for each plot of each trial for both disease incidence and severity. Calculation of *AUDPC* used the midpoint method of Campbell and Madden (1990), where *AUDPC* is estimated by the formula:

$$AUDPC = \sum_{i}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Equation 4.3

where y_i was the disease level (incidence or severity) at assessment time i, y_{i+1} was the disease level in the subsequent time period, t_i was the time (in days) at the i^{th} time and t_{i+1} was the time at the subsequent period. Spatial association, in the form of co-occurrence, was then measured using SADIE (Winder *et al.* 2001) between the disease measurements (disease incidence and severity) and crop measurements (plant density, capsule dry matter yield, alkaloid percentage yield and total alkaloid yield,

the product of alkaloid yield and capsule yield). To enable assessment of co-occurrence the spatial pattern of *AUDPC* values for disease incidence and severity and for each crop factor were first assessed using SADIE. For analysis, all values were rounded to integer values. Alkaloid content was multiplied by a factor of 100 prior to rounding to prevent the loss of significance in values, while both total alkaloid yield and capsule yield were assessed as grams per total quadrat area. The maximum 9999 randomisations were conducted for all analyses. A two-tailed test was used with a null hypothesis of no association against the alternatives of spatial association, indicated bypositive values of X, or spatial dissociation indicated by negative values of X (Winder *et al.* 2001).

As an alternative measure of relationship between crop factors and *AUDPC* values over both seasons, Pearson's correlations were calculated using Genstat software (version 5; Lawes Agricultural Trust, Rothamsted, UK). The calculated correlation co-efficient (r) was then compared to published critical values (Steel & Torrie 1980) for level of significance.

Spatial association and Pearson's correlation was also measured between crop factors and disease measurements at the individual time periods. In these instances, the spatial pattern of disease incidence and severity for each individual time period was compared to spatial pattern of each crop factor using SADIE. Correlations were calculated using measured disease incidence and severity at each time period. Spatial association and Pearson's correlation were also measured between plant density and the individual crop yield components (alkaloid content and capsule dry matter yield), and total alkaloid yield.

4.3. Results

4.3.1. Temporal analysis

In 2000, the proportion of plants infected by downy mildew (incidence) increased from 0.09±0.003 (±standard error of mean) at 53 DAS, to 0.99±0.002 at 103 DAS (Fig. 4.1A). The proportion of leaf area affected (severity) increased from 0.005±0.0008 to 0.10±0.002 (Fig. 4.1B). In 2001, no infection was observed at 45 DAS, while incidence increased from 0.002±0.0009 at 55 DAS, to 0.998±0.0008 at 95 DAS (Fig. 4.1A), and severity increased from 0.00005±0.00002 to 0.12±0.01 (Fig. 4.1B).

Temporal changes in incidence were best described by the linear disease model (R^2 = 0.992) in 2000, and the logistic model (R^2 = 1.000) in 2001 (Fig. 4.1A). For disease severity, in both seasons the model of best fit was the exponential model with R^2 = 0.953 and R^2 = 0.994 for 2000 and 2001 respectively (Fig. 4.1B).

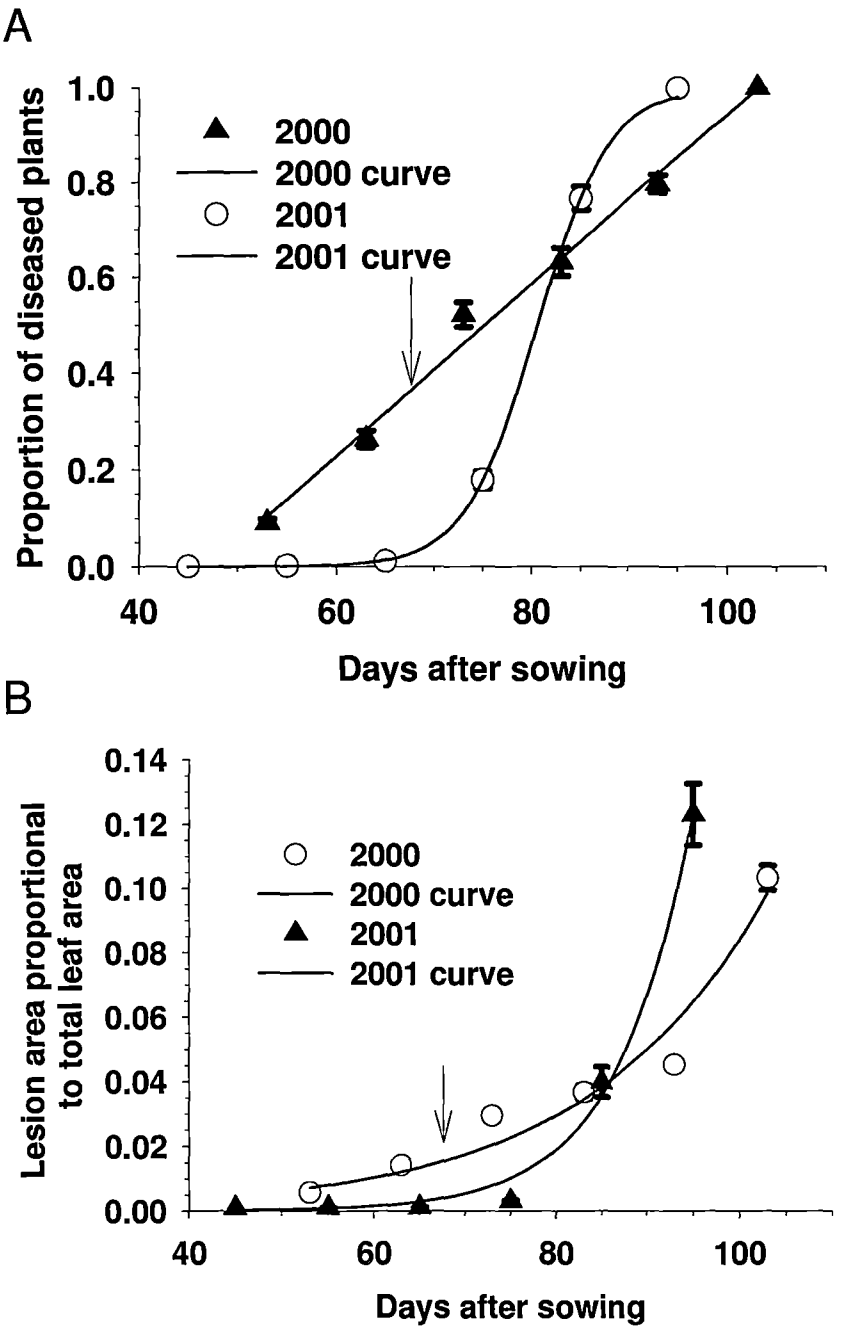


Fig. 4.1 Temporal change in incidence (A), and proportion of total leaf area affected (B) by downy mildew in 2000 and 2001. Arrows indicate the timing of fungicide application in 2000. Bars represent the standard error of the mean. Equations of curves for incidence (A) are y = -0.844 + 0.0179x in 2000 (R^2 = 0.992) and y = 1 / (1 + $3.58x10^{9}e^{-0.273x}$) in 2001 (R^2 = 1.00), and severity (B) are y = $4.45x\ 10^{-4}e^{0.0524x}$ in 2000 (R^2 = 0.953) and y = $8.70E\text{-}7e^{0\ 125x}$ in 2001 (R^2 = 0.994).

4.3.2. Spatial analysis

Spatial aggregation was detected by all spatial analyses for all data sets tested, with the exception of disease incidence at 53 DAS in 2000 (Table 4.2).

Distribution analysis for disease incidence in 2000 indicated that the beta-binomial distribution provided a significantly ($P < 0.001$) better fit than the binomial distribution for all assessments between 63 and 93 DAS (Table 4.2). At 53 DAS in 2000, there was no evidence to suggest that the beta-binomial distribution provided a better fit than the binomial distribution. Values of the index of dispersion, D, increased from 1.1 at 53 DAS to a maximum of 5.6 at 83 DAS, and then decreased to 3.2 at 93 DAS (Table 4.2). In 2001, disease incidence at 75 and 85 DAS was better described by the beta-binomial distribution than the binomial ($P < 0.001$). Values of D increased from 6.3 at 75 DAS to 6.4 at 85 DAS (Table 4.2).

Using median runs analysis, significant spatial aggregation was detected in incidence in 2000 along rows (running east to west) at 63 ($P < 0.05$), 73, 83 and 93 DAS ($P < 0.001$; Table 4.2). For incidence, significant aggregation along columns (running north to south) was detected at 73 ($P < 0.1$), 83 and 93 ($P < 0.05$) DAS. For severity, significant aggregation was detected both along rows ($P < 0.001$) and along columns ($P < 0.01$) at 103 days. In 2001, significant aggregation for incidence was detected along columns (north to south) at 75 ($P < 0.01$) and 85 DAS ($P < 0.001$), while significant ($P < 0.01$) aggregation along rows (east to west) was detected at 85 DAS (Table 4.2). For severity at 95 DAS, significant aggregation was detected along columns ($P < 0.001$) and along rows ($P < 0.01$). In 2000, the Z-statistics for aggregation of incidence decreased from 0.44 (both along rows and along columns)

at 53 DAS to minimums of –4.4 (along rows) and –2.14 (along columns) at 83 DAS, and then increased to –3.3 (along rows) and –2.07 (along columns) at 93 DAS (Table 4.2). In 2001, the *Z*-statistics for aggregation of incidence decreased from –2.5 (along columns) and –1.2 (along rows) at 75 DAS to –4.9 (along columns) and -2.5 (along columns) at 85 DAS (Table 4.2).

Significant I_a values indicating aggregation were obtained by SADIE analysis for incidence at 63, 73, 83 and 93 DAS and for severity at 103 DAS in 2000 (Table 4.2). In 2001, aggregation was indicated by I_a at 75 and 85 DAS for incidence and 95 DAS for severity. For incidence in 2000, I_a reached a maximum at 63 DAS of 2.24 (Table 4.2). For incidence in 2001, I_a increased from 1.81 at 75 DAS to 2.98 at 85 DAS (Table 4.2). The index of aggregation under crowding, J_a, was only valid at 53 and 83 DAS for incidence in 2000, and 75 DAS for incidence and 95 DAS for severity in 2001. In all these cases J_a was in agreement with I_a (Table 4.2).

The spatial position of the disease foci was identified by plotting the clustering indices output from SADIE for each period in which spatial aggregation was detected (Fig. 4.2 and Fig. 4.3). For incidence at 63 DAS in 2000, patch clusters (areas of significantly high incidence) were situated at the eastern end of the trial site with a gap cluster (area of significantly low incidence) at the western end (Fig. 4.2A). For incidence at 73, 83 and 93 DAS, patch clusters were present in the centre of the trial site with a gap cluster at the western end (Fig. 4.2B to D). For severity at 103 DAS in 2000, patch clusters were present in the centre of the field with a gap cluster at the eastern end (Fig. 4.2E). For 2001, patch clusters were present in the northern and central areas of the trial site with gap clusters in the south-eastern corner, at all time periods for both incidence and severity (Fig. 4.3A to

C). For severity at 95 DAS, a second gap cluster was present at the western end of the trial site (Fig. 4.3C).

Table 4.2 Spatial analyses of incidence and severity of downy mildew of oilseed poppy at intervals during the 2000 and 2001 seasons, using spatial analysis by distance indices (SADIE), distribution fitting and median runs analysis.

Season	Data type	Days after sowing[1]	SADIE		Distribution fitting		Median runs	
			I_a[2]	J_a[2]	LRS[2]	D[2]	Column runs[2]	Row runs[2]
2000	Incidence	53	0.714	0.999	-0.084	1.078	0.4408	0.4408
		63	2.24****[3]	-[4]	-22.04****	2.266	0.1429	-1.858**
		73	1.46*	-	-81.05****	3.930	-1.277*	-3.280****
		83	1.43*	1.05**	-159.3****	5.626	-2.135**	-4.425****
		93	1.55**	-	-59.92****	3.239	-2.071**	-3.280****
	Severity	103	1.74**	-	n.a.[5]	n.a.	-3.001***	-3.572****
2001	Incidence	75	1.81**	1.08*	-110.5****	4.282	-2.533***	-1.182
		85	2.98****	-	-261.1****	6.444	-4.911****	-2.489***
	Severity	95	2.44****	1.32****	n.a.	n.a.	-5.198****	-2.458***

[1]time periods where the overall incidence (or severity) was < 5% or > 95% were not assessed

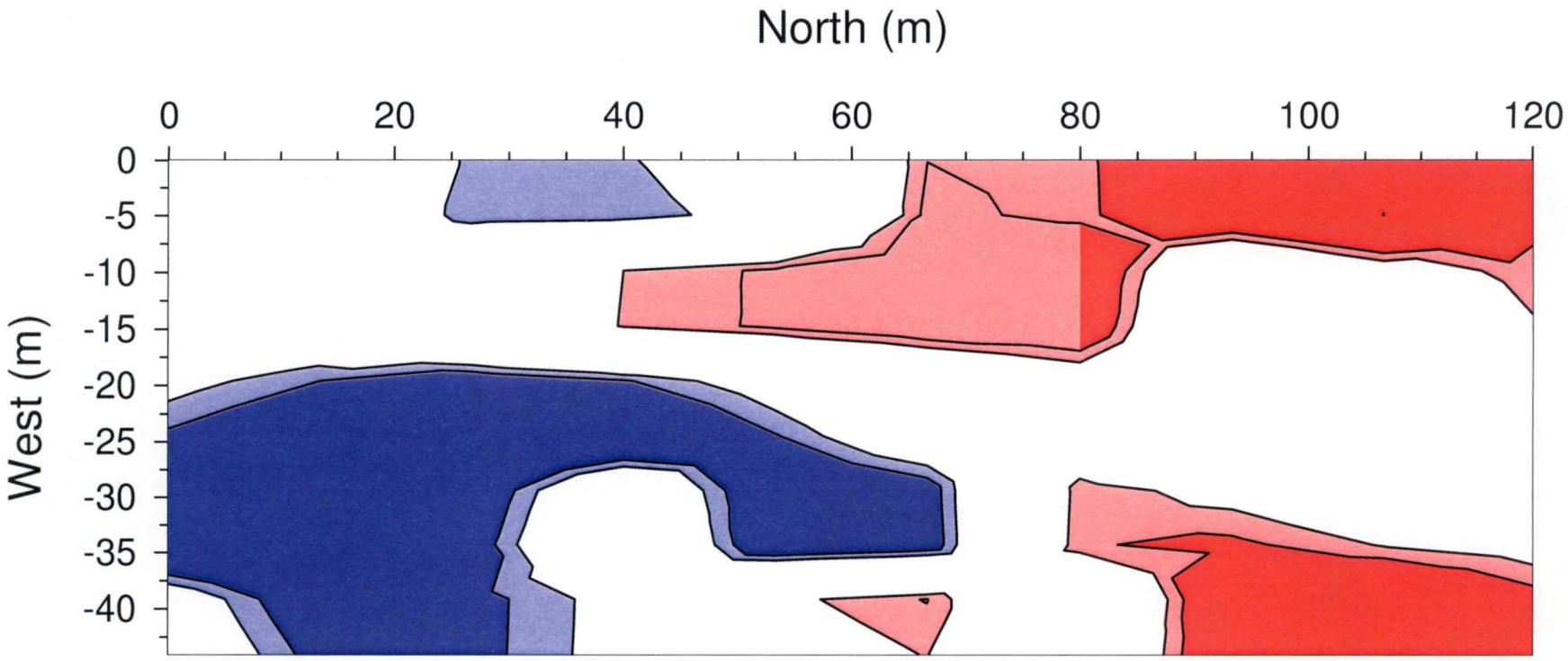
[2]I_a and J_a are the indices of aggregation under clustering and crowding respectively; LRS is the log-likelihood ratio test statistic and D is the index of dispersion; column runs and row runs are the Z statistics of observed against expected

[3]level of significance * = $P < 0.1$, ** = $P < 0.05$. *** = $P < 0.01$, **** = $P < 0.001$

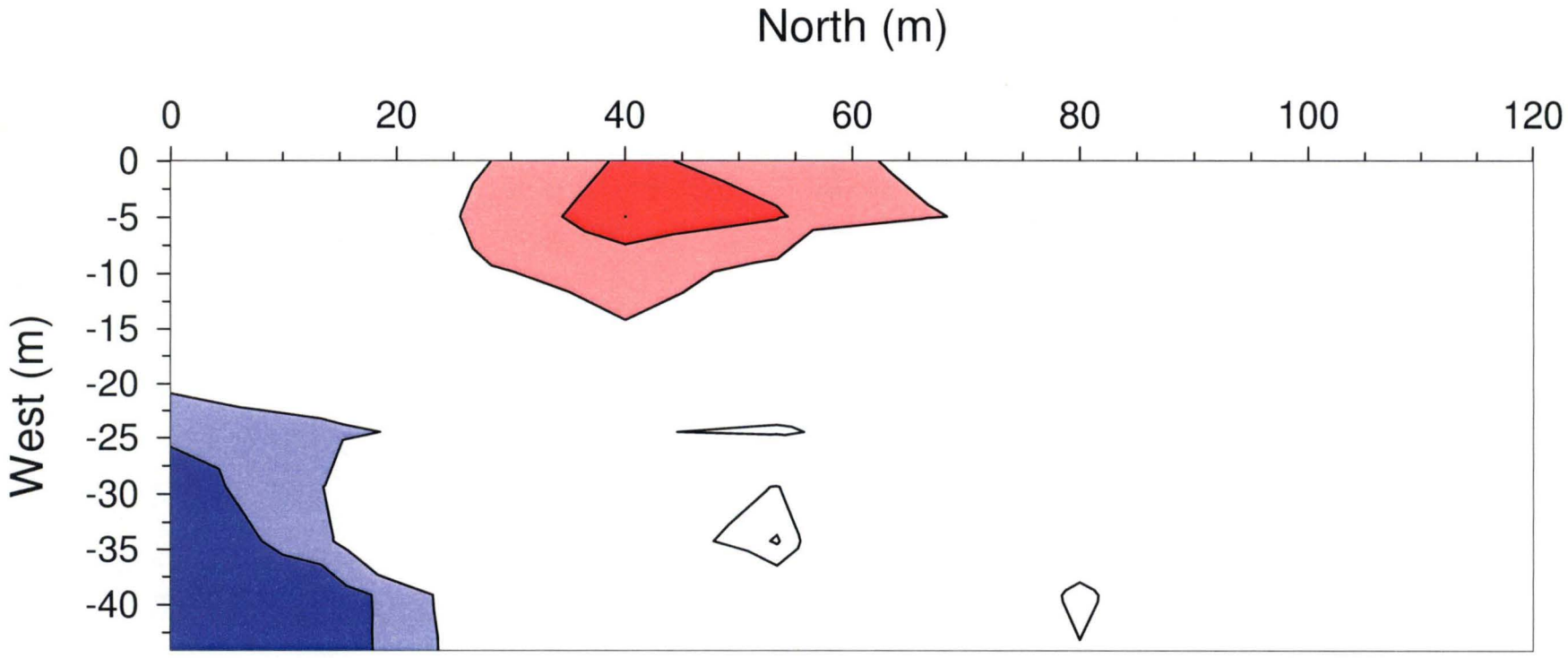
[4]J_a, listed when 1 or less disease patches present in a data set

[5]not applicable as distribution fitting and indices of dispersion are only applicable to incidence (binary) data.

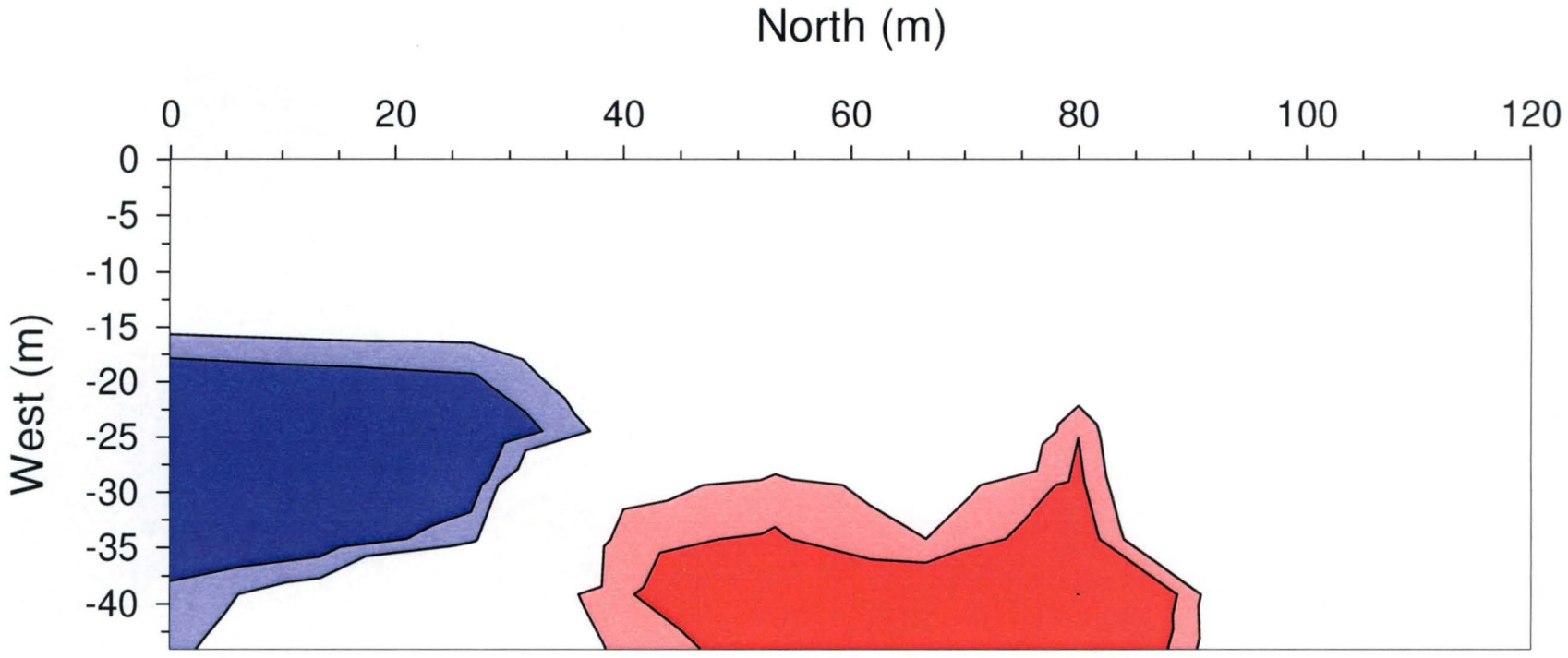
A



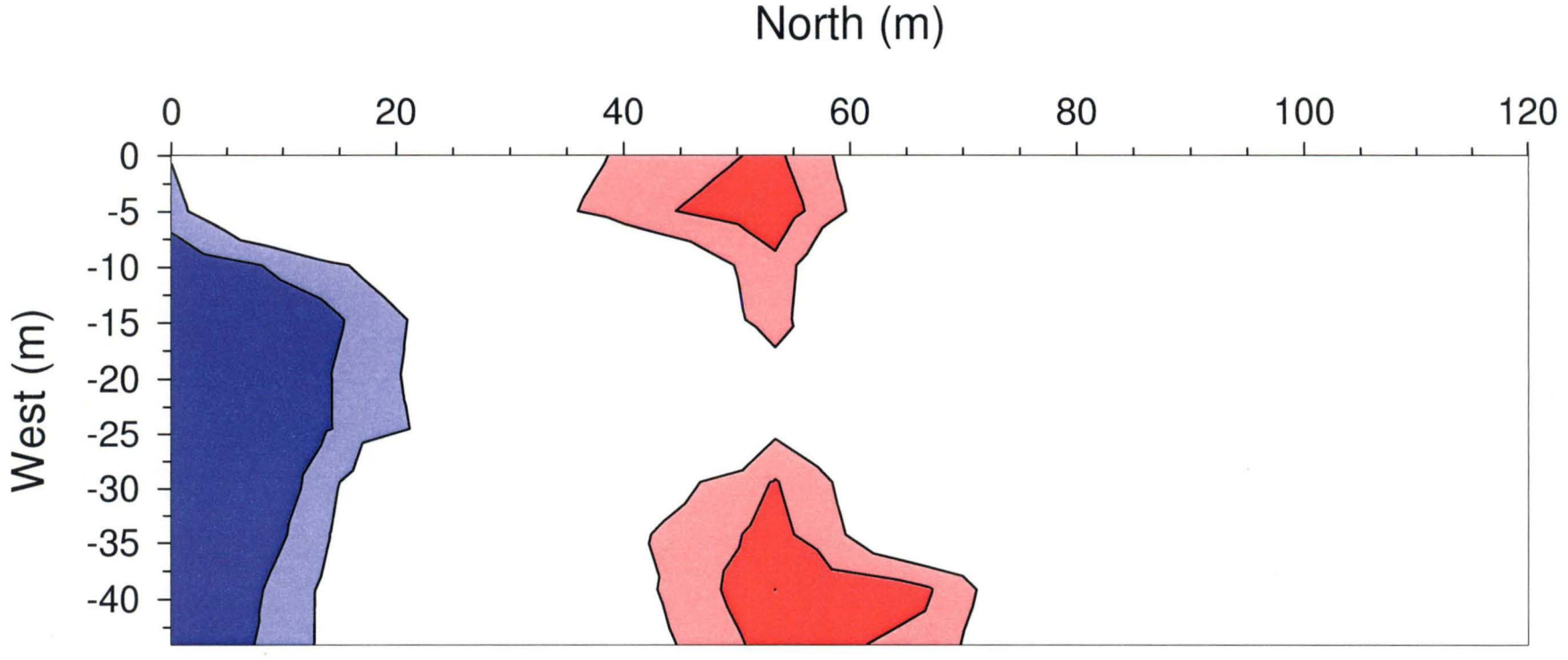
B



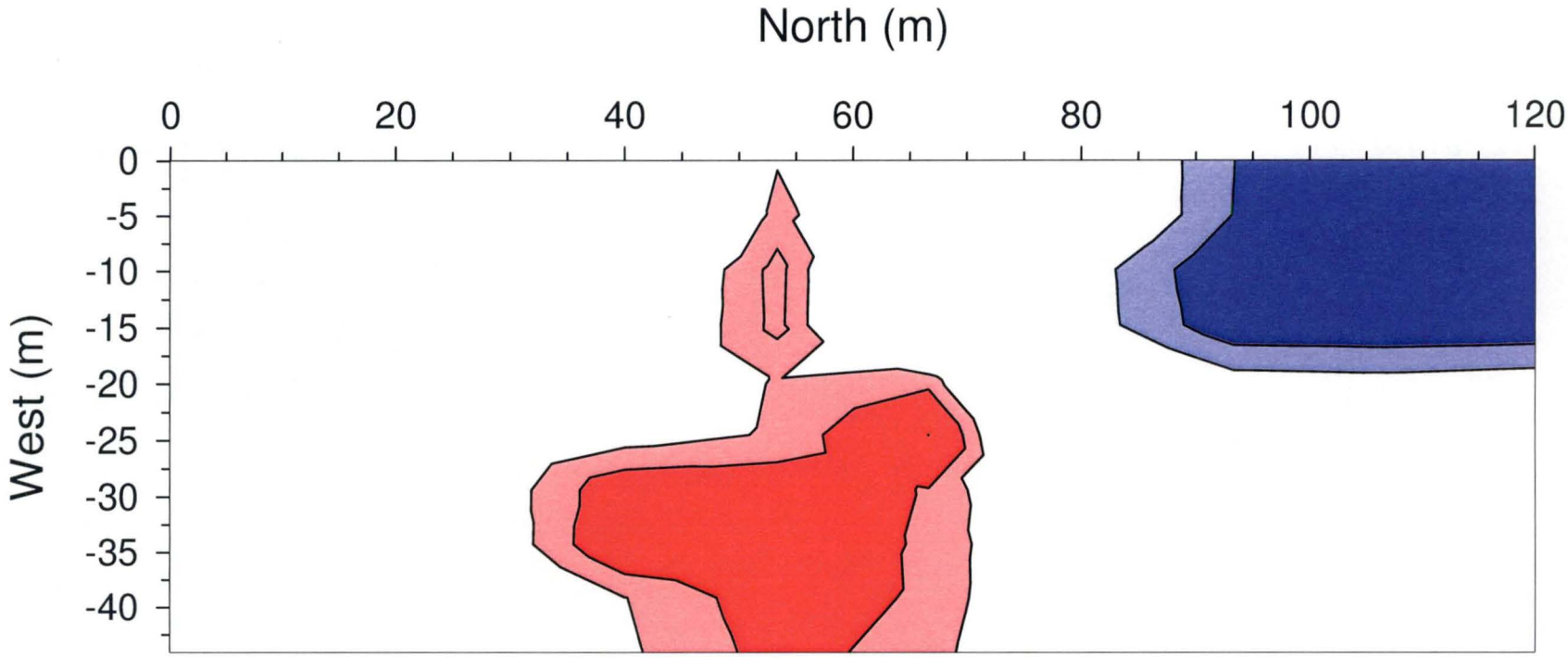
C



D



E



Key to Fig. 4.2A-E

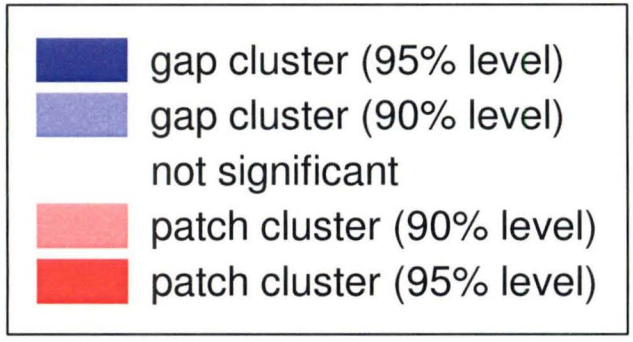
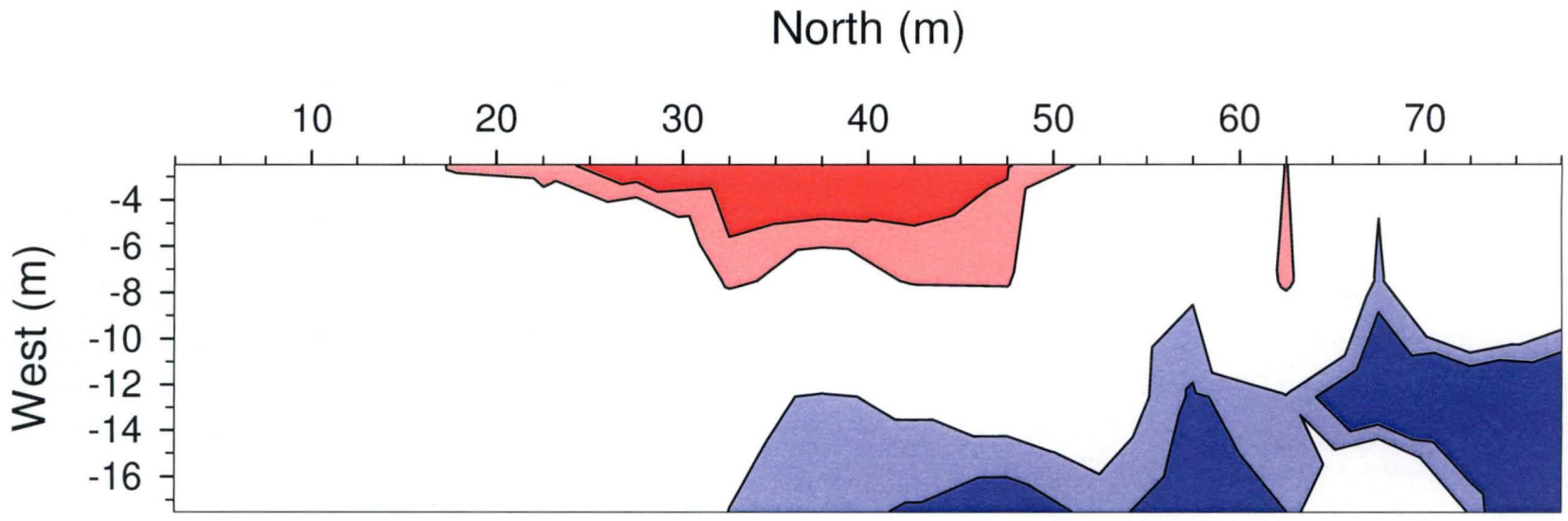


Fig. 4.2 Contour plots of SADIE clustering indices for the 2000 season for, A) incidence at 63 days after sowing (DAS), B) incidence at 73 DAS, C) incidence at 83 DAS, D) incidence at 93 DAS, and E) severity at 103 DAS. Gap clusters defined as areas with $v_j < 90^{th}$ or 95^{th} percentile of v_j values under randomization, where v_j is the clustering index of sampling units with values less than the field mean. Patch clusters defined as areas with clustering indices $> 90^{th}$ or 95^{th} percentile of v_i values under randomization, where v_i is the clustering index of sampling units with values greater than the field mean.

A



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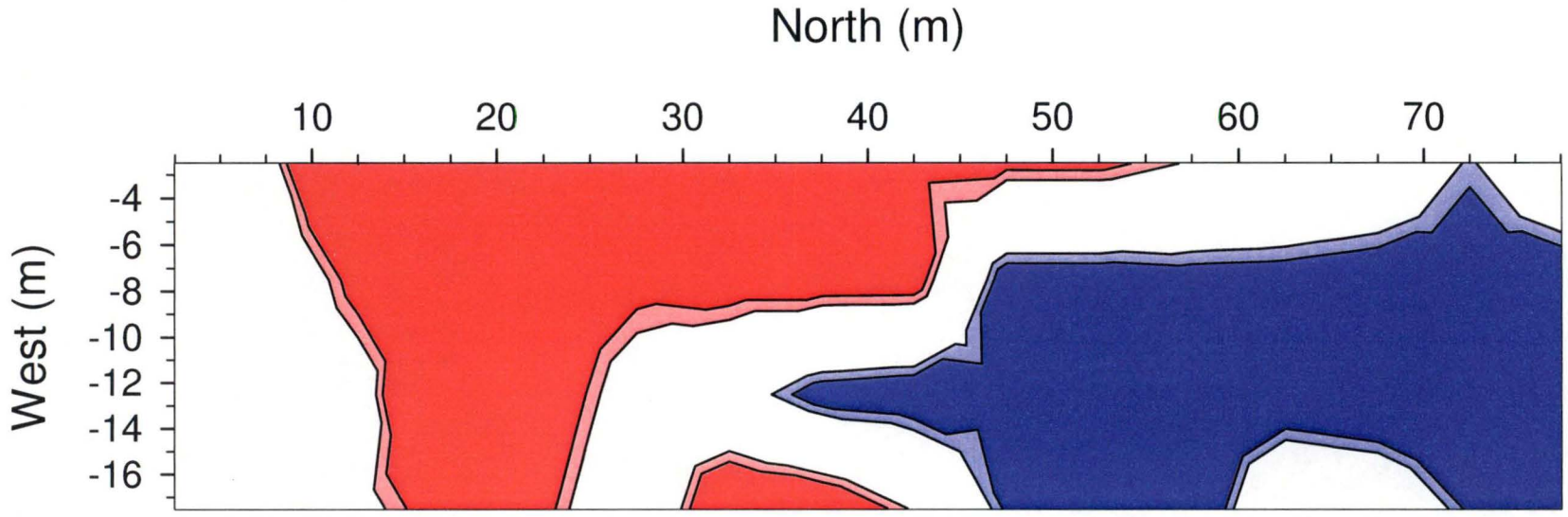
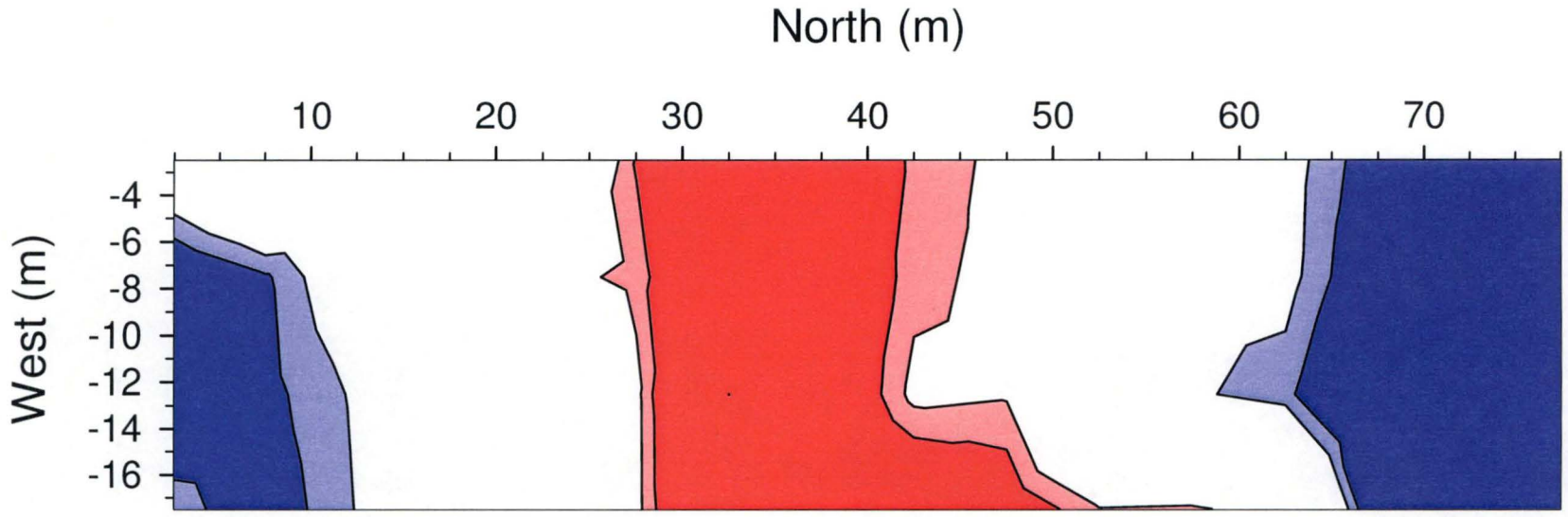


C



Key to Fig. 4.3A-C

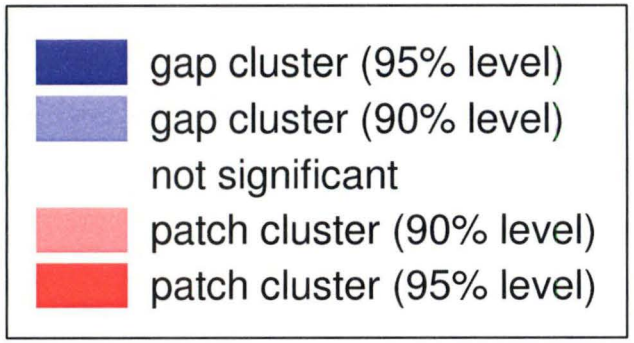


Fig. 4.3 Contour plots of SADIE clustering indices for the 2001 season for, A) incidence at 75 days after sowing (DAS), B) incidence at 85 DAS, and C) severity at 95 DAS. Gap clusters defined as areas with $v_j < 90^{th}$ or 95^{th} percentile of v_j values under randomization, where v_j is the clustering index of sampling units with values less than the field mean. Patch clusters defined as areas with clustering indices $> 90^{th}$ or 95th percentile of v_i values under randomization, where v_i is the clustering index of sampling units with values greater than the field mean.

4.3.3. Spatiotemporal analysis

Strong significant spatial associations ($P < 0.005$) were detected between all those time periods which exhibited spatial aggregation and the successive time period in each season (Table 4.3). Significant spatial association was also detected between 63 and 83 DAS for incidence ($P < 0.025$), 63 and 93 DAS for incidence ($P < 0.05$) and 73 and 93 DAS for incidence ($P < 0.005$) in 2000 (Table 4.3). Strongly significant spatial association was also detected between disease incidence at 93 DAS and disease severity at 103 DAS in 2000 ($P < 0.005$). No significant spatial association was observed between the random spatial pattern of disease incidence at 53 DAS and any of the subsequent time periods in 2000 (Table 4.3). In 2001, significant spatial association was observed between 75 DAS for incidence and 95 DAS for severity ($P < 0.05$), and 85 DAS for incidence and 95 DAS for severity ($P < 0.005$).

Table 4.3 Overall association indices (X) from spatial analysis by distance indices (SADIE) spatiotemporal analysis of the 2000 and 2001 growing seasons.

		Incidence				Severity
2000	**DAS[1]**	**63**	**73**	**83**	**93**	**103**
Incidence	**53**	0.0592	-0.0159	-0.0579	0.113	0.0108
Incidence	**63**	-	0.384***[2]	0.343**	0.239*	-0.181
Incidence	**73**	-	-	0.497****	0.597***	0.211
Incidence	**83**	-	-	-	0.689****	0.265
Incidence	**93**	-	-	-	-	0.501***

		Incidence	Severity
2001	**DAS**	**85**	**95**
Incidence	**75**	0.846****	0.277*
Incidence	**85**	-	0.435***

[1]days after sowing

[2]level of significance of X under a two-sided test for association (positive values of X) or dissociation (negative values of X); * = $P <$ 0.05, ** = $P < 0.025$, *** = $P < 0.005$, **** = $P < 0.0005$

4.3.4. Spatial association

Spatial aggregation ($P < 0.05$) of alkaloid content as a percentage of capsule dry matter was detected by SADIE in both 2000 and 2001 (Table 4.4). In 2001, spatial aggregation was also detected in plant density ($P < 0.001$), capsule dry matter yield ($P < 0.05$) and total alkaloid yield ($P < 0.05$). No spatial aggregation was detected in plant density, capsule dry matter yield or total alkaloid yield in 2000. Spatial aggregation was detected in *AUDPC* values for disease incidence and severity in 2000 ($P < 0.05$ and $P < 0.1$ respectively) and 2001 (both $P < 0.001$; Table 4.5).

Table 4.4 Summary of crop measurements taken for each quadrat in the 2000 and 2001 growing seasons, including output of spatial analysis of data using SADIE.

		min.	**mean**	**max.**	**s.e.m.**[1]	I_a[2]
plant density	2000	50.8	69.8	91.8	0.461	1.16
(plants.m^{-2})	2001	18.7	36.6	67.3	0.462	3.21 ****[3]
capsule yield	2000	185.5	308.2	391.5	0.979	0.741
(g.m^{-2})[4]	2001	128.9	198.9	301.7	0.822	1.89 **
Alkaloid content	2000	1.18	1.58	1.89	0.049	1.53 **
(% dry matter)	2001	1.28	2.17	2.58	0.064	1.79 **
Total alkaloid yield	2000	2.20	4.89	6.64	0.1345	0.690
(g.m^{-2})[4]	2001	2.78	4.28	6.46	0.123	1.81 **

[1]standard error of mean

[2]index of aggregation under regularity

[3]level of significance under one-sided test for aggregation, ** = $P < 0.05$, **** = $P < 0.001$

[4]SADIE and correlation analyses conducted on capsule yield and total alkaloid yield as measured as grams per total quadrat area

Table 4.5 Spatial analyses of area under disease progress curve values for disease incidence and severity in the 2000 and 2001 seasons, SADIE.

Season	Disease incidence		Disease severity	
	I_a[1]	P[2]	I_a	P
2000	1.71	**	1.47	*
2001	2.79	****	2.55	****

[1]index of aggregation under regularity

[2]level of significance under a one-sided test for aggregation * = $P < 0.1$, ** = $P < 0.05$, **** = $P < 0.001$

Spatial association and positive correlation ($P < 0.005$) were detected between *AUDPC* values and plant density in the 2001 season (Table 4.6). No association or correlation was detected in 2000. Spatial association and positive correlation ($P < 0.005$) were detected between capsule yield and *AUDPC* severity values in 2001 (Table 4.6). No other significant relationships between capsule yield and *AUDPC* values were detected in either season. Spatial dissociation ($P < 0.005$) was detected between *AUDPC* values and alkaloid contents in both seasons (Table 4.6). Negative correlation ($P < 0.005$) occurred between *AUDPC* values and alkaloid contents in 2000, but not in 2001 (Table 4.6). Spatial dissociation ($P < 0.025$) was detected between total yield and *AUDPC* severity values in 2000, but not negative correlation (Table 4.6). Positive correlation ($P < 0.025$) was detected between total yield and *AUDPC* severity values in 2001, but not spatial association (Table 4.6). No other significant relationships between total yield and *AUDPC* values were detected.

Spatial dissociation was detected between plant density and both alkaloid content and total alkaloid yield in 2000 ($P < 0.025$) and 2001 ($P < 0.005$; Table 4.7). No significant spatial association was detected between capsule yield and plant density

in either season. No significant correlations were detected between plant density and any of the crop yield factors in either season (Table 4.7).

Table 4.6 Pearson's correlation co-efficient (*r*) and overall association index (*X*) from SADIE association between area under disease progress curves for disease incidence and severity and crop yield factors in the 2000 and 2001 growing seasons.

Season		Statistic	Plant density	Capsule yield	Alkaloid content	Total yield
2000	incidence	*r*	-0.105	0.077	-0.447 ***[1]	-0.088
		X	-0.112	0.194	-0.398 ***	-0.211
	severity	*r*	-0.118	0.104	-0.387 ***	-0.043
		X	-0.0883	0.106	-0.410 ***	-0.294 **
2001	incidence	*r*	0.456 ***	0.202	-0.059	0.168
		X	0.485 ****	0.148	-0.625 ****	0.0277
	severity	*r*	0.369 ***	0.453 ***	-0.235	0.299 **
		X	0.477 ****	0.345 ***	-0.673 ****	0.171

[1]level of significance under two-sided test for a) positive correlation (positive values of *r*) or negative correlation (negative values of *r*); and b) association (positive values of *X*) or dissociation (negative values of *X*); ** = $P < 0.025$, *** = $P < 0.005$, **** = $P < 0.0005$

Table 4.7 Pearson's correlation co-efficient (*r*) and overall association index (*X*) from SADIE association, between plant density and crop yield factors in the 2000 and 2001 growing seasons.

Season	Statistic	Capsule yield	Alkaloid content	Total alkaloid yield
2000	*r*	-0.015	-0.110	-0.063
	X	-0.122	-0.317 **[1]	-0.310 **
2001	*r*	-0.158	-0.125	-0.158
	X	-0.139	-0.394 ***	-0.371 ***

[1]level of significance under two-sided test for a) positive correlation (positive values of *r*) or negative correlation (negative values of *r*); and b) association (positive values of *X*) or dissociation (negative values of *X*); ** = $P < 0.025$, *** = $P < 0.005$

In 2000, no significant spatial association or Pearson's correlation were detected between plant density and disease incidence, or severity, at any time period (Table 4.8). In 2001, significant spatial association was detected between plant density and disease incidence at 75 ($P < 0.005$) and 85 DAS ($P < 0.005$), and between plant density and disease severity at 95 DAS ($P < 0.025$; Table 4.9). Significant positive correlations between plant density and disease incidence were detected at 65 ($P < 0.025$), 75 ($P < 0.005$) and 85 DAS ($P < 0.025$), and disease severity at 65 ($P < 0.025$), 75 ($P < 0.005$) and 85 DAS ($P < 0.005$).

In 2000, no significant Pearson's correlation was detected between capsule yield and disease incidence, or severity, at any time period (Table 4.8). SADIE detected a single significant spatial association between disease incidence at 73 DAS and capsule yield (Table 4.9). In 2001, significant spatial association occurred between capsule yield and disease severity at 95 DAS ($P < 0.025$; Table 4.8). Significant positive correlations occurred between capsule yield and disease incidence at 85 DAS ($P < 0.025$), and disease severity at 95 DAS ($P < 0.005$).

In 2000, significant spatial dissociations occurred between alkaloid percentage of dry matter and disease incidence at 73 ($P < 0.025$), 83 ($P < 0.005$) and 93 DAS ($P < 0.025$). Significant negative correlations occurred between alkaloid percentage and disease incidence at 83 ($P < 0.005$) and 93 DAS ($P < 0.005$), and disease severity at 83 ($P < 0.005$) and 93 DAS ($P < 0.005$). In 2001, significant spatial dissociations occurred between alkaloid percentage and disease incidence at 75 ($P < 0.005$) and 85 DAS ($P < 0.0005$), and disease severity at 95 DAS ($P < 0.0005$). A significant negative correlation occurred between alkaloid percentage and disease severity at 95 DAS ($P < 0.025$).

In 2000, significant spatial dissociations between total alkaloid yield and disease incidence occurred at 83 ($P < 0.05$) and 93 DAS ($P < 0.025$). No significant correlation was detected between total alkaloid yield and disease in 2000. In 2001, a significant spatial association occurred between total alkaloid yield and disease severity at 95 DAS ($P < 0.0005$). Significant positive correlations occurred between total alkaloid yield and disease severity at 55 DAS ($P < 0.025$), and disease severity at 55 ($P < 0.025$) and 95 DAS ($P < 0.025$).

Table 4.8 Pearson's correlation co-efficient (r) and overall association index (X) from spatial analysis by distance indices (SADIE) association, results of analyses between crop factors and disease incidence or severity over the 2000 growing season.

		Incidence						Severity					
	Statistic	**53 DAS[1]**	**63 DAS**	**73 DAS**	**83 DAS**	**93 DAS**	**103 DAS**	**53 DAS**	**63 DAS**	**73 DAS**	**83 DAS**	**93 DAS**	**103 DAS**
Plant density	*R*	-0.201	0.014	-0.081	-0.085	-0.151	0.017	-0.133	0.010	-0.109	-0.096	-0.227	0.020
	X	-0.2104	-0.0349	-0.0707	-0.1301	-0.0092	n.a.[3]	n.a.	n.a.	n.a.	n.a.	n.a.	0.0145
Capsule yield	*R*	-0.076	0.117	0.156	-0.039	0.056	0.126	-0.086	0.116	0.181	-0.020	-0.018	0.170
	X	0.1038	0.1939	0.3682 **[2]	0.1188	0.1434	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.1049
Alkaloid content	*R*	-0.212	-0.140	-0.304	-0.476 ***	-0.436 ***	-0.190	-0.194	-0.131	-0.286	-0.471 ***	-0.421 ***	-0.039
	X	-0.0139	-0.2084	-0.2896 **	-0.3588 ***	-0.3419 **	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-0.0415
Total alkaloid yield	*R*	-0.133	0.052	0.029	-0.203	-0.098	0.040	-0.134	0.055	0.049	-0.189	-0.155	0.149
	X	0.0179	0.0573	-0.0160	-0.2450 *	-0.3119 **	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-0.0089

[1]days after sowing

[2]level of significance under two-sided test for a) positive correlation (positive values of r) or negative correlation (negative values of r); and b) association (positive values of X) or dissociation (negative values of X); * = $P < 0.05$, ** = $P < 0.025$, *** = $P < 0.005$

[3]not assessed

Table 4.9 Pearson's correlation co-efficient (r) and overall association index (X) from spatial analysis by distance indices (SADIE) association, results of analyses between crop factors and disease incidence or severity over the 2001 growing season.

		Incidence						Severity					
	Statistic	**45 DAS[1]**	**55 DAS**	**65 DAS**	**75 DAS**	**85 DAS**	**95 DAS**	**45 DAS**	**55 DAS**	**65 DAS**	**75 DAS**	**85 DAS**	**95 DAS**
Plant density	*R*	n.a.	-0.002	0.337 **[2]	0.446 ***	0.371 **	-0.231	n.a.[3]	-0.002	0.329 **	0.474 ***	0.454 ***	0.160
	X	n.a.	n.a.	n.a.	0.3494 ***	0.4157 ***	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.2032 **
Capsule yield	*R*	n.a.	0.277	0.111	-0.044	0.328 **	0.266	n.a.	0.277	0.072	-0.070	0.193	0.591 ***
	X	n.a.	n.a.	n.a.	0.0716	0.1786	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.5835 ***
Alkaloid content	*R*	n.a.	0.051	-0.064	0.141	-0.195	-0.051	n.a.	0.051	0.032	0.081	-0.085	-0.327 **
	X	n.a.	n.a.	n.a.	-0.3215 ***	-0.5609 ****	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-0.5520 ****
Total alkaloid yield	*R*	n.a.	0.310 **	0.090	0.047	0.209	0.232	n.a.	0.310 **	0.106	-0.010	0.140	0.375 **
	X	n.a.	n.a.	n.a.	0.0120	0.0927	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.4646 ****

[1]days after sowing

[2]level of significance under two-sided test for a) positive correlation (positive values of r) or negative correlation (negative values of r); and b) association (positive values of X) or dissociation (negative values of X); ** = $P < 0.025$, *** = $P < 0.005$, **** = $P < 0.0005$

[3]not assessed

4.4. Discussion

To the author's knowledge, this is the first study using temporal and spatial analysis techniques to quantify the development of downy mildew epidemics in oilseed poppy.

Temporal analysis of disease development suggested that epidemics are best described by polycyclic disease models (Madden 1980). Disease severity was best described by the exponential curve in both seasons. In 2001, temporal progression of disease incidence was defined by a typical logistic curve, whilst in 2000 a linear curve provided the best fit. This inconsistency between seasons may be attributed to the fungicide application at 67 DAS in 2000. Visual observation of the 2000 curve suggests that the epidemic may have been in, or entering, the exponential phase of development when the fungicide was applied, with the resultant effect of suppressing disease spread. This change in disease dynamics delayed disease incidence reaching a maximum in the course of this study. Observation of the 2000 severity curve also revealed a flattening of the curve after fungicide application.

With the exception of the linear increase in disease incidence in 2000, observation of the disease progress curves showed rapid development in downy mildew epidemics following canopy closure (63 and 65 DAS in 2000 and 2001 respectively). Incidence of downy mildew has also been closely correlated with the stage of crop growth in lettuce (Wu *et al.* 2001b). This may be attributed to the microclimatic changes occurring in the crop at different growth stages. Canopy closure in poppies would result in increased humidity within the crop allowing the development of greater levels of secondary inoculum.

The temporal results of this study also highlight the ability of the pathogen to propagate itself rapidly under favourable conditions. In uncontrolled conditions in 2001, disease incidence, as a proportion, increased from 0.002 to 1.0 in a period of 40 days, a greater than 500 fold increase. This is consistent with the results obtained for onion downy mildew (*Peronospora destructor* (Berk.) Casp.), where incidence increased from 0.00 to 1.0 in approximately 43 days, or four infection cycles (Hildebrand & Sutton 1982).

In both seasons, all spatial analysis methodologies agreed when indicating either aggregated or random spatial patterns for all data sets assessed. Spatial aggregation was detected in assessments conducted after canopy closure. Prior to canopy closure in 2000, a random spatial pattern was observed. This dynamic nature to spatial pattern has also been observed for other downy mildews, where the early stages of epidemics exhibit random distribution or low levels of aggregation, increasing spatial aggregation until mid-epidemic, and then exhibit decreases in aggregation at high disease incidences (Madden *et al.* 1995; Stein *et al.* 1994). This same pattern was observed in D under distribution fitting and Z under median runs analysis, in 2000 incidence data, but not in I_a with SADIE. The decrease in the level of aggregation detected by SADIE following the application of a fungicide, in contrast to the results of median runs analysis and distribution fitting, suggested that SADIE may be more sensitive to aggregation than these other forms of analysis. In 2001, where no fungicide application occurred, all three analyses indicated increasing aggregation of disease incidence from 75 to 85 DAS.

Spatiotemporal analysis using SADIE demonstrated that following the development of spatial aggregation within a field, the spatial pattern of disease in one time period was strongly associated with the spatial pattern of the previous time period. This strong association between spatial patterns suggested that new disease foci were not developing within the field over time, or were of lesser importance to epidemic progress than already established foci. This suggested that localised secondary spread, presumably in the form of conidia (Yossifovitch 1929), is the predominant mechanism behind epidemic development.

Both Pearson's correlation and spatial association analysis indicated a positive relationship between plant density and disease in 2001. Examination of disease at specific time periods showed that this relationship occurred post-canopy closure. Pearson's correlation indicated a positive relationship at 65, 75 and 85 DAS for both disease incidence and severity whilst spatial association occurred at 75 and 85 DAS for disease incidence and 95 DAS for disease severity. These results indicate that disease was favoured by increasing plant densities, especially following canopy closure, which is consistent with a disease favouring high relative humidities (Behr 1956; Yossifovitch 1928). These results were not replicated in the 2000 season, although this may be due to the lack of spatial aggregation observed in the plant density in this season, and thus insufficient differences between the the individual plant densities of quadrats to examine differences in disease.

Alkaloid content, as a percentage of harvested capsule dry matter, exhibited spatial dissociation with disease incidence and severity over both seasons. In 2000, Pearson's correlation indicated a significant negative relationship with both disease incidence and severity at 83 and 93 DAS, while SADIE indicated a significant

dissociation with disease incidence at 73, 83 and 93 DAS. In 2001, significant dissociations occurred with disease incidence at 75 and 85 DAS and disease severity at 95 DAS, while a negative correlation also occurred with disease severity at 95 DAS. The timing of all of these negative relationships coincides with the development of bolting through to late capsule formation. Previous studies have shown there is a marked increase in the level of alkaloid accumulation within oilseed poppies from bolting until capsule formation (Williams & Ellis 1989). These results therefore indicate that disease stress due to downy mildew during this time period can significantly reduce alkaloid production. This agrees with previous work that has detected significant negative correlations between opium weight and disease (Nigam *et al.* 1989). The principal means of this reduction would be through the reduction in plant photosynthetic area by downy mildew lesions (Bernath & Nemeth 1998). Plant density may also have affected alkaloid content as a negative correlation was also detected between alkaloid content and plant density. Chung (1990) showed that alkaloid percentages were reduced above the optimum plant density of 70 plant.m^{-2}. However in 2001, plant densities in this study were below this density suggesting that reduced alkaloid content was related to disease, rather than plant density. In addition, the significance of the dissociation between disease and alkaloid content under SADIE was as strong, or stronger, than the dissociation between plant density and alkaloid content for disease incidence and severity in both seasons. It is probable that the dissociation of alkaloid content and plant density was an artefact of high plant densities favouring high disease levels, and high disease levels reducing alkaloid production.

No consistent relationship between capsule yield and disease was found in this study. Spatial association and positive correlation was detected for disease severity in 2001,

but for all other cases no relationship was detected. Examination of the timing of association showed sporadic associations, and positive correlations, over both seasons. No negative relationships between disease and capsule yield were detected. This suggests that disease may be favoured by the denser plant growth associated with high capsule yields, but no negative effect of disease on dry matter yield occurred in this work.

As total yield is the product of its components (alkaloid content and capsule yield) the differing relationships of these components to disease have led to an inconsistent relationship between total alkaloid yield and disease, with both positive and negative relationships observed over the two seasons. A more detailed yield study incorporating average individual capsule weights may be necessary to elucidate the effect of disease on these yield components.

In all, 58 occasions where both SADIE spatial association and the Pearson's correlation co-efficient could be calculated occurred over both seasons. On 12 occasions spatial association detected a significant relationship where no significant correlation occurred, whilst there were only two instances when significant correlations, but no spatial association, were recorded. No instances of the two analyses indicating significant, conflicting relationships occurred. These results indicate general agreement between the two analyses, which is to be expected as the SADIE spatial association statistic, X, is the Pearson's correlation co-efficient of clustering indices of the two data sets in question (Perry & Dixon 2002). The apparent greater sensitivity of SADIE is due to spatial association measuring the correlation between the spatial pattern of two data sets, and is directly dependant on the magnitude of the respective values (Perry & Dixon 2002).

The evidence collected in this study suggested that the predominant mechanism in epidemic development for downy mildew in oilseed poppy is localised secondary spread, principally after canopy closure. However microclimatic conditions, which are influenced by crop development, also play a role in regulating spread. These results suggest that during crop growth the control measures should be targeted towards controlling secondary, rather than primary, spread, after the onset of canopy closure. In addition, cultural techniques such as reduced crop density could be considered for downy mildew control.

5. Modelling the weather conditions affecting downy mildew epidemics

5.1. Introduction

Epidemics caused by downy mildew pathogens, especially those of the genus *Peronospora*, are known to be influenced by climatic conditions. Previous research on the downy mildew pathogen of oilseed poppy (recorded as *P. arborescens*) has shown that climatic factors, including temperature, relative humidity, free moisture and light, can influence epidemic development. Temperature has been recorded as effecting both conidium survival (Behr 1956; Kothari & Prasad 1970) and conidium germination (Behr 1956; Yossifovitch 1929). High relative humidity has been recorded to favour both sporulation (Kothari & Prasad 1970; Yossifovitch 1928) and conidium germination (Behr 1956; Doshi & Thakore 1993; Yossifovitch 1929). Kothari and Prasad (1970) found that free moisture was required for a period of at least four hours to enable conidium infection, specifically germ tube elongation (Doshi & Thakore 1993). Light is known to inhibit sporulation (de Weille 1961), while also influencing conidium survival and germination, with small doses of UV light promoting germination, but larger doses increasing conidium mortality (de Weille 1961; Doshi & Thakore 1993).

Understanding how specific environmental conditions affect epidemics can be beneficial for the development of control strategies. If the effect of climate is understood, cultural techniques can often be employed to prevent favourable disease conditions. Such cultural techniques include alteration of sowing dates to avoid favourable climatic conditions for epidemic development, or modification of the crop morphology to prevent microclimatic conditions favouring epidemics. Another

alternative for disease control is weather monitoring and/or prediction, which can then be used for the strategic application of chemical fungicides to maximise their effectiveness and minimise their usage.

The ultimate extension of weather monitoring for the strategic application of fungicides is the development of predictive models. To the author's knowledge, no predictive models have been previously developed, or utilized, for poppy downy mildew epidemics. However, three distinct models have been described for the prediction of epidemics involving *Peronospora* spp; DOWNCAST (Jesperson & Sutton 1987), ONIMIL (Battilani *et al.* 1996a; Battilani *et al.* 1996b), and ZWIPERO (Friedrich *et al.* 2003). All three models have been developed for the prediction of onion downy mildew (*P. destructor*). DOWNCAST was first developed for the prediction of epidemics in Canada (Jesperson & Sutton 1987), and has subsequently been adapted further to conditions in the Netherlands (de Visser 1998). ONIMIL and the recently described ZWIPERO represent adaptations to DOWNCAST that attempt to quantify the relative levels of infection predicted.

The purpose of this study was to model the epidemic development of *P. cristata* relative to environmental conditions. The predictive model, DOWNCAST, as described by Jesperson and Sutton (1987), and modified by de Visser (1998), was validated for its predictive power for *P. cristata* epidemics.

5.2. Materials and methods

5.2.1. Culture maintenance

Downy mildew infected leaves were collected from field surveys and incubated to promote sporulation in sealed containers with damp tissues at 12 °C and 100 % relative humidity for 12 hours under dark conditions. Following incubation, conidia were removed from leaves by shaking in distilled water (dH_2O). Conidium suspensions were adjusted to 10^4 to 10^5 conidia.mL^{-1} with the aid of a haemocytometer. Healthy *Papaver somniferum* plants were grown from seed under glasshouse conditions (23±0.1/15±0.1 °C and 73±0.4/87±0.3 % relative humidity, day/night ±standard error of the mean) in potting mix (Appendix V). Plants at the 10 leaf stage (Appendix III) were infected by wiping the leaves with cotton wool to remove surface wax and misted with 2 mL of conidium suspension. To promote infection, host plants were incubated overnight in a misting chamber under glasshouse conditions, with regular fine mists of water applied to maintain leaf wetness. Mists were applied for 10 s at five minute intervals during daylight hours, and at 30 min intervals under dark conditions. After incubation, infected plants were maintained under glasshouse conditions. Routine maintenance was carried out fortnightly, by harvesting symptomatic leaves, incubating at 12 °C and 100 % relative humidity overnight and rubbing sporulating leaves on fresh host plants. Fresh plants were then incubated in a misting chamber to promote infection, as previously described.

5.2.2. Weather monitoring

All weather monitoring was conducted using Watchdog™ 450 dataloggers (Spectrum Technologies Inc., Plainfield, ILL, USA). Readings were taken of temperature, relative humidity, rainfall, and leaf wetness at 15 min intervals. All readings were taken with sensors placed within crop canopies at 10 cm above ground level.

Temperature and relative humidity were recorded using the internal sensors of Watchdog dataloggers. According to company specifications temperature and relative humidity readings had accuracies of ±0.7 °C and ±3 % respectively.

Rainfall was monitored with external, continuous flow through, Compact Rainfall Sensors (Spectrum Technologies). Sensors measured rainfall at increments of $1/100^{th}$ of an inch, with an accuracy of ±4 %.

Leaf wetness was monitored with an external Leaf Wetness Sensor (Spectrum Technologies). Sensors were flat electrical resistance grids with a reading range of 0.0 (dry) to 15.0 (wet).

5.2.3. Laboratory studies of sporulation

To study the effect of temperature and relative humidity on the sporulation of the downy mildew pathogen, laboratory studies of sporulation under controlled conditions were conducted.

Healthy plants at approximately the 10 leaf stage (Appendix III) were inoculated as per culture maintenance. Following incubation in a misting chamber, plants were maintained under dry glasshouse conditions (22±0.1/14±0.1 °C and 64±0.6/79±0.4 % relative humidity, day/night respectively) for seven days. Leaf disks (2 cm diameter) were cut from infected leaves and used for studies of the effect of temperature on sporulation. Seventy leaf disks were randomly allocated to one of seven different temperature treatments (5, 10, 15, 20, 25, 30 °C and control). For treatments 5, 10, 15, 20, 25 and 30 °C ten leaf disks were randomly allocated to two sealed containers (containing five disks each) with damp tissues to maintain 100 % relative humidity. Containers were then incubated for 12 hours under dark conditions at their respective temperatures. Infected control disks were placed in two open containers without damp tissue and left at room temperature for 12 hours. Following incubation, containers were opened and individual disks scored for the presence of sporulation.

A second temperature study was conducted to determine the optimum temperature for sporulation at 100 % relative humidity. Leaf disks were produced as previously described. Based on the results of the first study, 120 leaf disks were randomly allocated over six temperature treatments (4, 8, 12, 16, 20 and 24 °C). For each treatment, 20 disks were randomly allocated to four sealed containers with damp tissue to maintain 100 % relative humidity. Containers were then incubated for 12 hours under dark conditions at their respective temperatures. A further 20 disks were placed in open containers without damp tissue and left at room temperature for 12 hours as a control. Following incubation, disks were shaken in sterile distilled water (SDW) to dislodge conidia produced. Conidium suspensions were concentrated by centrifugation at 3000 *g* (r_{av} 139 mm) for 20 min at room temperature, removal of the supernatant by pipetting and then resuspending in 100 µL of SDW. Conidium

concentrations were determined with the aid of a haemocytometer. Due to low levels of conidium production, samples were combined into a single sample for each treatment and concentrated into 100 µL of SDW as previously described. Conidium concentration for each treatment was then determined by three replicate counts of conidia using a haemocytometer.

The interaction of relative humidity and temperature on sporulation was investigated by varying the osmotic potential of water agar to control relative humidity (Appendix VI; Harris *et al.* 1970; Lang 1967). Twenty millilitres of 1 % agar (Powdered Agar Grade J3, Leiner Davis Gelatin (Australia), QLD, Australia), containing varying concentrations of sodium chloride (Appendix VI), was poured into petri plates and used as controlled atmosphere containers. Infected plants were generated as previously described. Infected leaves were harvested and cut into thirds. Eight relative humidity (87, 89, 91, 92, 95, 97, 99 and 100 %) and six temperature (4, 8, 12, 16, 20 and 24 °C) treatments were tested. Six leaf pieces were randomly allocated to each relative humidity/temperature combination, with three leaf pieces each per plate. Leaf pieces were sealed within petri plates with plastic film and incubated at their respective temperatures for 12 hours in the dark. An additional six leaf pieces were placed in two open plates without agar, as controls. After incubation individual leaf pieces were scored for the presence of sporulation.

5.2.4. Field monitoring of sporulation

Monitoring of sporulation events was conducted during the 2001/2002 (2001) and 2002/2003 (2002) growing seasons. Monitoring took place within one field crop of poppies in the 2001 season, and two field crops in the 2002 season. Field crops were

visually surveyed early season for the presence of downy mildew. Monitoring of sporulation began subsequent to downy mildew incidence increasing above 5 %. In 2001, monitoring was conducted between the 17th November and the 16th December. In 2002, monitoring was conducted in two different crops between the 10th and 23rd December at crop 1, and the 3rd and 23rd December at crop 2. All field crops were situated at the TAFE-Freer Farm, Burnie (S41° 04', E145° 51'; Appendix I). Weather readings were taken throughout the monitoring period, as described previously.

Sporulation events were detected through the placement of healthy oilseed poppy (trap) plants, generated as per culture maintenance, within the crop canopy near infected plants. Ten plants were placed daily within field crops and exposed for a single day. All plants were placed and removed from crops during late afternoon. After removal from the crop, trap plants were incubated within a misting chamber under glasshouse conditions, overnight with the same misting regime as per culture maintenance, to induce infection of trap plants by conidia present. Following incubation, plants were maintained for 14 days under dry glasshouse conditions and basal watered to prevent leaf wetness. Plants were harvested and incubated in sealed trays at 12 °C and 100 % relative humidity for 12 hours and examined at 10x magnification for the presence of sporulation. A single plant exhibiting sporulation was considered an indication of a sporulation event for that trapping day. For all days an additional two plants, which had not been placed in the field were maintained under dry glasshouse conditions as a control to check for any background levels of infection occurring in the glasshouse.

Weather data were analysed using the onion downy mildew forecasting model DOWNCAST (Jesperson & Sutton 1987), including the modifications of de Visser (1998; Fig. 5.1). Predicted occurrences of sporulation were compared to observed sporulation events in the field and the significance of model fit was compared using a chi-squared analysis of a two by two contingency table. A Yates corrected chi-squared value was calculated when the expected value of any particular cell was less than five.

5.2.5. Field monitoring of infection

Monitoring of infection events was conducted during the years 2001 and 2002. Infection events were recorded by artificially inoculating trap plants, generated as per culture maintenance, and placing them in an external environment. Infected leaves were harvested from culture and incubated to induce sporulation as previously described. Sporulating leaves were then used to dust 16 healthy plants at approximately the 10 leaf stage (Appendix III) with conidia by rubbing sporulating leaves on trap plants. One heavily sporulating leaf was used to inoculate two healthy plants. Plants were then placed at midday in an external environment with four uninoculated control plants. At 24 hour intervals, four plants were removed and placed in dry glasshouse conditions and watered at the base of pots to prevent further leaf wetness. After four days, control plants were also returned to the glasshouse. Plants were maintained in the glasshouse for 14 days, followed by harvest of the whole plant and incubation in a sealed tray to induce sporulation as previously described. Plants were then observed at 10x magnification and scored for the presence of sporulation. The first day in a four day period observed to produce greater than a single sporulating lesion (over four plants) was deemed to contain a

period of infection. Weather monitoring was conducted during each inoculation and exposure period, as described previously. A four day monitoring period was chosen to encompass the assumption of conidium survival for a maximum of three days used in DOWNCAST (Jesperson & Sutton 1987).

Weather data collected were analysed using the onion downy mildew forecasting model DOWNCAST (Fig. 5.1). To account for differences in scale between Watchdog Leaf Wetness Sensors and those sensors employed by de Visser (1998) it was necessary to recalculate the critical limits of leaf wetness for infection and conidium death. Visual field observations comparing leaf wetness sensor readings with actual leaf wetness within the canopy of poppy crops suggested that crops were wet above sensor readings of 6.0. To account for a degree of error, the critical sensor reading indicating the onset of leaf wetness (WETLIMIT; Table 5.1) was thus set at the mid point of the sensor range (7.5). In keeping with de Visser (1998), the minimum sum of leaf wetness values over a five hour period to ensure rapid dew formation (SURF1; Table 5.1) was calculated using an assumed linear increase in leaf wetness values over the five hour period from the minimum value indicating a deviation from dry leaves (arbitrarily set at 1.0) to the critical limit for infection (1.0, 2.625, 4.25, 5.875 and 7.5 respectively). This resulted in a value of 21.25 for SURF1. Similarly the minimum sum of leaf wetness values over the subsequent three hour period to ensure infection (SURF2; Table 5.1) was set at 22.5 (three times WETLIMIT). In keeping with the settings outlined by de Visser (1998), the critical total of leaf wetness values over a five hour period indicating conidium death (MORT; Table 5.1) was set at 60 % of SURF1 (12.75). Predicted occurrences and timing of infection were compared to observed infection events and the significance of model fit was compared using chi-squared analysis of a two by two contingency

table. A Yates corrected chi-squared value was calculated when the expected value of any particular cell was less than five.

5.2.6. Model development

The individual parameters and constraints of DOWNCAST were examined empirically to validate their effect on prediction. Modified parameters and constraints were then combined into a modified model for the prediction of downy mildew epidemics. To avoid confusion this model is referred to as POPCAST (an acronym for POPpy downCAST). POPCAST was built in a stepwise progression, with the order of parameter analysis based on the assumed relative importance of each parameter to sporulation, or infection, with the most important parameter used for initial model development. Subsequent parameters were then added to the initial model and empirically tested for improvements to prediction. Parameters improving prediction were maintained in the model structure, while parameters providing no improvement, or decreases in the accuracy of prediction, were discarded. The sporulation and infection sub-models of DOWNCAST were examined separately and then combined to produce the overall POPCAST prediction model. The timing of sunrise and sunset employed by DOWNCAST matched the actual occurrence of sunrise and sunset in early summertime under daylight savings time in Tasmania, and therefore were not altered.

A basic requirement for the sporulation of downy mildews is a nightime period of high relative humidity (Hildebrand & Sutton 1982; Kothari & Prasad 1970; Yarwood 1943; Yossifovitch 1928). Therefore the critical level of relative humidity (RHLIM; Table 5.1) and the length of time above this level were used as initial predictors of

observed sporulation. Values of RHLIM tested for sporulation were 94, 95, 96 and 97 %. The time lengths of the sporulation incubation period tested were 3, 4, 5, 6 and 7 hours. In conjunction, predictions based on continuous relative humidity above the critical limit were compared to the alternative, total number of hours above the critical limit during the night period. Night temperature requirements for sporulation were then examined based on the outcomes of the initial relative humidity results. After determination of the critical temperature limits for sporulation, incorporation of the effect of temperature on the length of the relative humidity period required for sporulation as outlined by de Visser (1998) was tested for improvement to prediction. Following this the critical limit for the total rainfall during the sporulation period (RAINLIM; Table 5.1) was determined. In this case, the more conservative definition of the sporulation period, between 0:00 and 6:00, of de Visser (1998) was used rather than the period 1:00 to 6:00 defined by Jesperson and Sutton (1987). Finally, the effect of high temperatures the preceding day, as outlined by de Visser (1998), was tested.

Initial development of the infection sub-unit of POPCAST was based on the required critical limit for leaf wetness using Watchdog™ Leaf Wetness Sensors. Critical limits of the sum of leaf wetness over five and three hours respectively, indicating rapid dew formation (DEW1) and subsequent infection (DEW2), were calculated as per DOWNCAST. Initial values of DEW1 (21.25) and DEW2 (22.5) were based on a critical leaf wetness value of 7.5, and were thus the equivalent of SURF1 and SURF2 described previously (Table 5.1). Alternative values for DEW1 and DEW2 were calculated at decreasing 0.5 increments of the critical leaf wetness value (WETINF; Table 5.1). As visual field observations have indicated that the leaves of poppy crops are wet above values of 6.0, critical values greater than 7.5 were not

tested. The critical sum of leaf wetness values indicating slow dew deposition leading to conidium death (WETMORT; Table 5.1) was then tested by setting values equal to 25, 50, 75 and 100 % of DEW1. Finally, the upper and lower critical temperature limits were determined based on observed infections.

Infection due to extended leaf wetness during the morning of conidium formation was not studied in this work. Therefore to predict infection in these instances the time period of four hours required for infection recorded by Kothari and Prasad (1970) was used. The value of WETINF and temperature constraints used was the same as those determined for infection due to dew deposition.

To provide an intermediate between DOWCAST and POPCAST, a second alternative model, termed modDOWNCAST (modified DOWNCAST) was also developed to evaluate prediction of epidemics. This model incorporated into the original DOWNCAST model only those parameters from POPCAST that were considered to provide significant improvement to the prediction of DOWNCAST. Parameter changes in POPCAST, relative to DOWNCAST, which improved prediction by only one or two time periods were not incorporated into modDOWNCAST.

Table 5.1 Description of key parameters used in onion downy mildew predictive model, DOWNCAST, and the equivalent parameter name employed in the alternative model, POPCAST.

DOWNCAST parameter[1]	**Description**	**POPCAST parameter**
HIGHRH	Minimum value of relative humidity favourable to sporulation	RHLIM
MORT	Maximum sum of leaf wetness values of five consecutive hours allowing for conidia to survive	WETMORT
RAINDARK	Maximum sum of rainfall between midnight and dawn still permitting sporulation to occur	RAINLIM
SURF1	Minimum sum of leaf wetness values of five consecutive hours ensuring rapid increase of leaf wetness values up to WETLIMIT	DEW1
SURF2	Minimum sum of leaf wetness values of three consecutive hours to ensure infection after rapid increase of leaf wetness values	DEW2
WETLIMIT	Minimum value indicating leaf wetness sufficient for infection to occur	WETINF

[1]DOWNCAST parameters and descriptions taken from de Visser (1998)

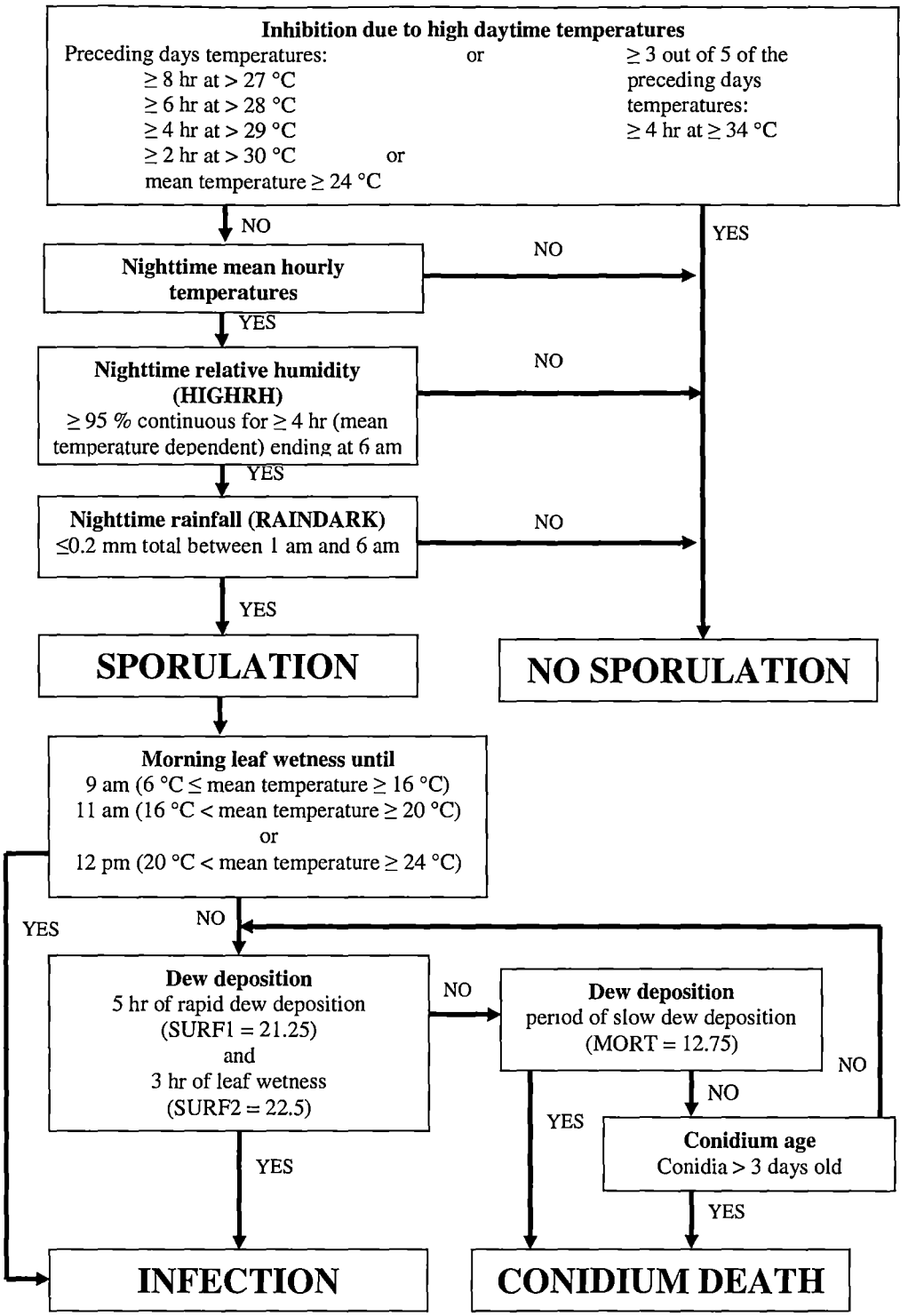


Fig. 5.1 Diagrammatic representation of the onion downy mildew forecaster model DOWNCAST as described by Jesperson and Sutton (1987) and modified by de Visser (1998). Nighttime is considered to be the hours 20:00 to 6:00. Daytime is considered to be between 6:00 and 20:00.

5.2.7. Model evaluation

To evaluate prediction models, the progression of disease incidence and severity were monitored in two commercial crops in the 2002 growing season. Both crops were situated on the TAFE-Freer Farm, Burnie (Table 5.2). Within each crop a 200 m^2 (40 by 50 m) area was marked out within the crop for sampling and was treated as part of the commercial crop. Disease incidence and severity were monitored from the 25th November to the 23rd December 2003, at seven day intervals. At each assessment period, one leaf from one of the bottom five nodes was arbitrarily sampled from 500 plants at each crop. Preliminary work had shown that these leaves were the most susceptible to infection (Appendix III). During the first four assessment periods, 100 samples were arbitrarily taken from each of five equal distance transects arranged in a 'zig-zag' pattern covering the entire sampling area. At the final assessment, period crop growth was such that transects could not be practically used for assessment. In this case, sampling was conducted along five spray runs (100 samples.run^{-1}), running parallel to one another through the sampling area. To provide greater coverage of the sampling area, leaves were sampled by reaching into the crop from the spray runs at arbitrary distances.

Following harvest, leaves were scored for the presence of downy mildew symptoms and for the severity of symptoms, using percentage leaf area diagrams (Appendix IV). Progression of disease incidence and severity (as proportions) was plotted for each crop by fitting standard disease progress curves to disease incidence and severity (Neher *et al.* 1997).

Weather monitoring was conducted one week prior to and throughout the sampling period, by placing Watchdog™ 450 dataloggers in the centre of the sampling area, as

described previously. Weather data were then analysed using DOWNCAST, POPCAST and modDOWNCAST. For model evaluation, predicted infection events were defined as the predicted infection of host plants by conidia formed by predicted sporulation events. A seven-day latent period, based on glasshouse observations, was assumed between the advent of infection as predicted by each model and the development of visual symptoms. The timing of predicted symptom development from each model was then overlayed with observed disease progression and compared visually. Preventative fungicide applications were assumed to have an active period of seven days, based on the recommended industry application schedule.

Table 5.2 Summary of agronomic activities pertinent to disease progression for crops used for disease monitoring for model evaluation in 2002 growing season at TAFE-Freer Farm, Burnie.

Site	Activity	Chemical[1]	Active constituent	Rate	Date (dd/mm/yy)
Crop 1	Sowing			900 $g.ha^{-1}$	28/09/02
	Fungicide	Dithane DF Agricultural Fungicide (Dow Agrosciences Australia, Ltd., NSW, Australia)	mancozeb, 750 $g.kg^{-1}$	2.5 $kg.ha^{-1}$	11/12/02
	Fungicide	Agri-Fos 600 Systemic Fungicide (Agrichem Manufacturing Industries Pty. Ltd., Queensland, Australia)	phosphonic acid, 600 $g.L^{-1}$	2 $L.ha^{-1}$	11/12/02
	Harvest				14/02/03
Crop 2	Sowing			900 $g.ha^{-1}$	29/09/02
	Fungicide	Dithane DF Agricultural Fungicide	mancozeb, 750 $g.kg^{-1}$	2.5 $kg.h^{-1}a$	10/12/02
	Fungicide	Agri-Fos 600 Systemic Fungicide	phosphonic acid, 600 $g.L^{-1}$	2 $L.ha^{-1}$	10/12/02
	Harvest				23/02/03

[1]if applicable

5.3. Results

5.3.1. Laboratory studies of sporulation

Sporulation was observed to occur in the temperature range 4 to 24 °C (Tables 5.3 & 5.4). In the first study of the effect of temperature, sporulation occurred at all temperatures tested between 5 and 20 °C (Table 5.3). No sporulation occurred at 25 or 30 °C or with the control. The greatest number of sporulating leaf disks (8 out of 10) occurred at 15 °C.

Table 5.3 Total number of sporulating *Papaver somniferum* leaf disks (out of 10) infected with *Peronospora cristata* after incubation at 100 % relative humidity for 12 hours at varying temperatures.

Temperature (°C)						
5	**10**	**15**	**20**	**25**	**30**	**control**
1	3	8	4	0	0	0

In the second study of the effect of temperature, sporulation was observed at all temperatures tested (Table 5.4). No sporulation occurred with the control. The greatest number of sporulating leaf disks occurred at the temperatures 16 and 20 °C (18 out of 20). The greatest number of conidia was produced at 16 °C with a mean of 867±233 (± standard error of the mean) conidia (Fig. 5.2). The least number of conidia were produced at 4 °C with a mean of 33±33 conidia (Fig 5.2).

Table 5.4 Total number of sporulating *Papaver somniferum* leaf disks (out of 20) infected with *Peronospora cristata* after incubation at 100 % relative humidity for 12 hours at varying temperatures.

Temperature (°C)						
4	**8**	**12**	**16**	**20**	**24**	**control**
1	8	15	18	18	12	0

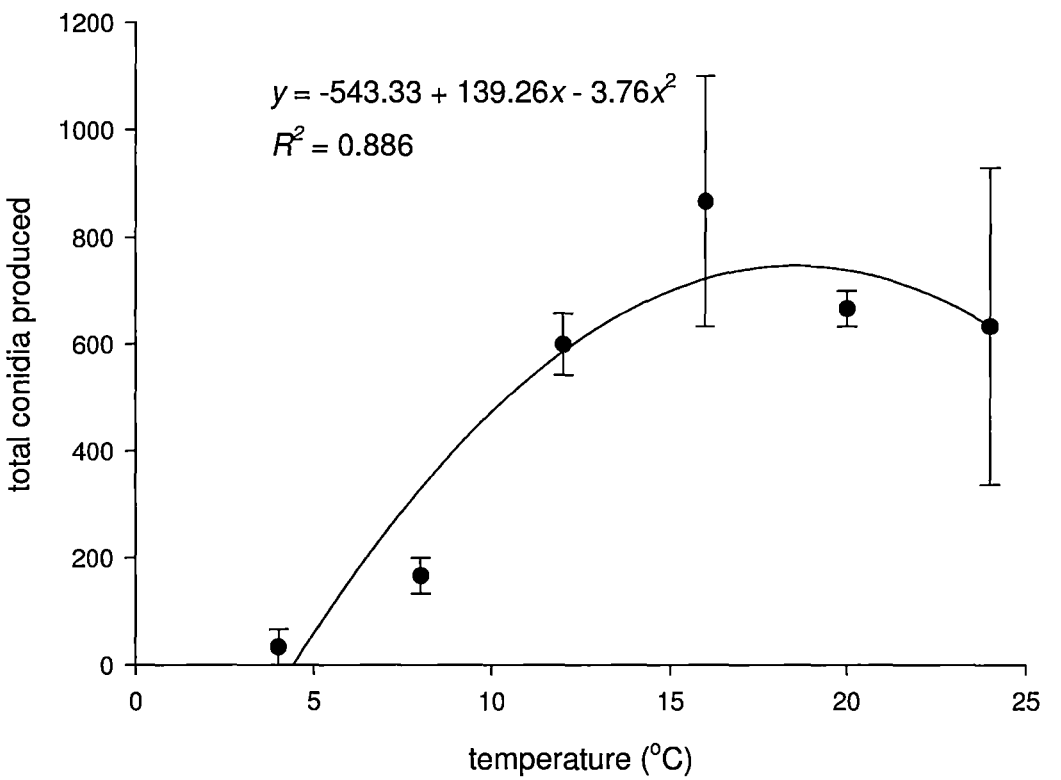


Fig. 5.2 Mean estimates of total number of conidia produced by twenty 2 cm diameter *Papaver somniferum* leaf disks infected with *Peronospora cristata* subjected to 100 % relative humidity for 12 hours at varying temperatures. Error bars represent the standard error of the mean of estimates.

No sporulation occurred at relative humidities 95 % and lower during the study of the interaction of relative humidity and temperature (Table 5.5). At 4 and 24 °C, sporulation only occurred at 100 % relative humidity. At 8 and 20 °C, sporulation occurred at 99 and 100 % relative humidity, while at 12 and 16 °C, sporulation

occurred at 97, 99 and 100 % relative humidity. The greatest number of sporulating leaf pieces (six out of six) was observed at 16 °C (99 % relative humidity) and 20 °C (99 and 100 % relative humidity; Table 5.5). The greatest number of total sporulating leaf pieces over all relative humidities (12 out of 42) occurred at 16 and 20 °C.

Table 5.5 Total number of sporulating *Papaver somniferum* leaf pieces (out of six), infected with *Peronospora cristata*, subjected to 12 hours incubation at varying temperatures and relative humidities.

Relative	**Temperature (°C)**					
humidity (%)	**4**	**8**	**12**	**16**	**20**	**24**
100	3	2	4	5	6	3
99	0	1	1	6	6	0
97	0	0	1	1	0	0
95	0	0	0	0	0	0
93	0	0	0	0	0	0
91	0	0	0	0	0	0
89	0	0	0	0	0	0

5.3.2. Field monitoring of sporulation

Out of a total of 63 trapping days, 37 instances of sporulation were recorded (Table 5.6; Appendices VIIA to VIIC). Of these 23 were predicted by DOWNCAST based on recorded weather data. Of the 26 instances of no sporulation detected, 24 of these were predicted by DOWNCAST. This resulted in an accuracy of prediction of sporulation for DOWNCAST of 75 %.

Table 5.6 Observed number of occurrences of sporulation and no sporulation compared to predicted occurrences, based on the disease forecaster model DOWNCAST.

		Observed		
		sporulation	**no sporulation**	**Total**
Predicted	**sporulation**	23	2	25
	no sporulation	14	24	38
	Total	37	26	63
$\chi_1^2 = 16.7$			$P < 0.001$	

Analysis of these results using a two by two contingency table indicated that the accuracy of prediction of DOWNCAST was significantly ($P < 0.001$) greater than expected under a random distribution of predictions (Table 5.6).

5.3.3. Field monitoring of infection

Out of a total of 20 infection monitoring periods, 12 infection events were recorded (Table 5.7; Appendix VIID). Of these, both the occurrence and timing of infection was correctly predicted by DOWNCAST for five periods, while in a further period infection was predicted, but on an incorrect day. All eight instances of no infection throughout the monitoring period were correctly predicted by DOWNCAST. This led to an overall accuracy of infection prediction by DOWNCAST of 65 %.

Chi-squared analysis of this data set was not valid due to the occurrence of an observed raw frequency of 0, and therefore was not undertaken. Visual observation of the data set suggested that the accuracy of prediction was not greater than would be expected under chance.

Table 5.7 Observed instances of infection and no infection compared to predicted timing of instances based on the DOWNCAST disease forecaster model.

		Observed		
		Infection	**no infection**	**Total**
Predicted	**Infection**	5	0	5
	No infection	7[1]	8	15
	Total	12	8	20
		not analysed[2]		

[1]includes one instance of predicted infection on an incorrect day

[2]χ^2 analysis not valid due to the occurrence of an observed raw frequency of 0

5.3.4. Model development

Sporulation

The maximum accuracy achieved using only relative humidity levels and length of high relative humidity period as predictors of sporulation was 52 out of 63 days (Table 5.8). This occurred with both total hours above RHLIM and continuous hours above RHLIM. With the requirement of continuous high relative humidity, a value of 96 % relative humidity for RHLIM for four hours provided optimum prediction (Table 5.8). Using total hours of high relative humidity, a value of 97 % for RHLIM and four hours was optimum (Table 5.8).

No mean nighttime temperatures occurred outside the range 4 to 24 °C, the initial DOWNCAST limits for sporulation (Appendices VIIA to VIIC).

When predictions were based on the interaction between temperature and the timing of the onset of high relative humidity as outlined in de Visser (1998), a maximum

accuracy of 50 out of 63 days was achieved. This level of accuracy occurred with RHLIM equal to 95 % relative humidity (Table 5.8).

Incorporation of the nighttime rainfall limit of 0.2 mm for the inhibition of sporulation resulted in a maximum accuracy of 50 out of 63 days. Increasing RAINLIM to 3 mm resulted in a maximum accuracy of predictions of 53 out of 63 days (Table 5.8). This occurred for both continuous relative humidity above 96 % for four hours, and a total of four hours above 97 % relative humidity.

Incorporation of the inhibition of sporulation due to high daytime temperatures the preceding day increased the optimum accuracy of POPCAST to 54 out of 63 days. No change in accuracy was observed from the incorporation of sporulation inhibition due to daytime temperatures greater than, or equal to 34 °C for four or more hours on three or more of the preceding five days. This occurred with RHLIM equal to 96 % relative humidity for a minimum of four hours using both continuous and total hours above this limit as predictors. It also occurred using a total of four hours above RHLIM, when RHLIM was equal to 97 % relative humidity. The maximum accuracy utilising the interaction of temperature and relative humidity (53 out of 63 days) occurred with RHLIM equal to 95 % relative humidity. Chi-squared analysis demonstrated that all predictions of sporulation based on the optimum combinations of relative humidity and inhibition due to both rainfall and high temperatures was greater than that expected by random chance (Tables 5.9 to 5.11). However, a total number of four hours above RHLIM equal to 97 % relative humidity produced the greatest value of chi-squared of 29.2 (Table 5.9).

Table 5.8 Total number of correct predictions of sporulation (out of 63) based on parameters used to develop POPCAST.

Predictor[1]	**Critical**	Total nighttime hours > critical limit					Continuous nighttime hours > critical limit					Temp / r.h.
	limit (%)	3	4	5	6	7	3	4	5	6	7	interaction[2]
Relative	**94**	45	46	46	46	46	45	45	48	47	46	48
humidity	**95**	45	47	49	51	46	46	49	50	51	47	50
	96	45	51	51	50	45	47	52	51	50	46	49
	97	50	52	47	45	37	49	51	47	43	36	45
Rainfall	**94**	46	47	47	47	47	46	46	49	48	47	49
inhibition (> 3.0	**95**	46	48	50	52	47	47	50	51	52	48	51
mm)	**96**	46	52	52	51	46	48	53	52	51	47	50
	97	51	53	48	46	38	50	52	48	44	37	45
Daytime high	**94**	49	50	50	50	48	49	49	51	50	48	51
temperature	**95**	49	51	52	53	48	49	52	52	53	49	53
inhibition[3]	**96**	49	54	53	52	46	50	54	53	52	47	50
	97	53	54	49	47	38	53	53	49	45	37	46

[1]parameters are cumulatively added to each other from top to bottom

[2]prediction of sporulation based on the interaction of the timing of the onset of high relative humidity and mean nighttime temperature as outlined by Jesperson and Sutton (1987)

[3]inhibition of sporulation due to high temperatures the preceding day as outlined by de Visser (1998)

Table 5.9 Chi-squared analysis of observed and predicted instances of sporulation. Predictions are based on a minimum total of four nighttime hours greater than a critical limit of 96 % relative humidity, and inhibition due to rainfall and high daytime temperatures.

		Observed		
		sporulation	**no sporulation**	**Total**
Predicted	**sporulation**	31	3	34
	no sporulation	6	23	29
	Total	37	26	63
$\chi_1^2 = 29.2$		$P < 0.001$		

Table 5.10 Chi-squared analysis of observed and predicted instances of sporulation. Predictions are based on a minimum total of four nighttime hours greater than a critical limit of 97 % relative humidity, and inhibition due to rainfall and high daytime temperatures.

		Observed		
		sporulation	**no sporulation**	**Total**
Predicted	**sporulation**	32	4	36
	no sporulation	5	22	27
	Total	37	26	63
$\chi_1^2 = 28.7$		$P < 0.001$		

Table 5.11 Chi-squared analysis of observed and predicted instances of sporulation. Predictions are based on a minimum of four continuous nighttime hours greater than a critical limit of 96 % relative humidity, and inhibition due to rainfall and high daytime temperatures.

		Observed		
		sporulation	**no sporulation**	**Total**
Predicted	**sporulation**	32	4	36
	no sporulation	5	22	27
	Total	37	26	63
$\chi_1^2 = 28.7$			$P < 0.001$	

Infection

Using a critical leaf wetness limit of 7.5, an accuracy of prediction of 10 out of 20 time periods was achieved (Table 5.12). Decreasing WETINF from 7.5 to 5.0 (resulting in totals of 15 for both DEW1 and DEW2) increased the accuracy of prediction of infection events to 11 out of 20 time periods.

The incorporation of a rule ensuring that hourly leaf wetness values did not fall below the minimum value indicating a deviation from dry leaf status (arbitrarily set at 1.0) during the DEW1 period, increased the accuracy of prediction using a leaf wetness limit of 7.5, to 11 out of 20 time periods (Table 5.12). This modification had no effect for other leaf wetness limits.

Incorporation of a critical limit to account for conidium death due to slow dew deposition increased the accuracy of prediction of POPCAST. The optimum value for WETMORT was observed at 100 % of DEW1. This occurred with critical leaf

wetness values of 4.5 and 5.0 and resulted in 16 correct predictions out of 20 time periods (Table 5.12).

Reincorporation of the upper (24 °C) and lower (6 °C) limits for infection resulted in the reduction of the optimum accuracy of infection prediction to 15 out of 20 time periods. This reduction was due to the occurrence of an infection event with an average temperature during this event of 2.0 °C on 17/6/02 (Appendix VIID). To account for this event, the lower limit for infection was reduced from 6 to 2 °C. No instances of infection occurring at temperatures above the upper limit for infection were recorded.

Using the above parameters, optimum and identical predictions occurred using both 4.5 and 5.0 as values of WETINF (Table 5.12). The lower value (4.5) was arbitrarily selected for use in the POPCAST infection sub-unit (Fig. 5.3).

modDOWNCAST

In the sporulation sub-unit of modDOWNCAST the only change made relative to the sporulation sub-unit of DOWNCAST was the setting of the value of RAINDARK, equal to that of RAINLIM employed in POPCAST (3 mm).

In the infection sub-unit of modDOWNCAST the value of WETLIMIT was set equal to WETINF (4.5) in POPCAST. In addition the values of SURF1, SURF2 and MORT were set equal to DEW1 (13.75), DEW2 (13.5) and WETMORT (13.75) in POPCAST, respectively.

Table 5.12 Total number of correct predictions (out of 20) of infection events based on parameters used to develop POPCAST.

Predictor[1]		Critical leaf wetness limit													
		1.0	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**	**4.0**	**4.5**	**5.0**	**5.5**	**6.0**	**6.5**	**7.0**	**7.5**
Leaf wetness		11	11	11	11	11	11	11	11	11	10	10	10	10	10
Continuous leaf wetness		11	11	11	11	11	11	11	11	11	10	10	10	10	11
WETMORT[2]	**100 % DEW1**	11	12	12	12	12	12	15	16	16	15	14	14	14	14
	75 % DEW1	10	12	12	12	12	12	12	12	12	14	14	14	14	14
	50 % DEW1	10	12	12	12	12	12	12	12	12	12	11	11	11	11
	25 % DEW1	10	12	12	12	12	12	12	12	12	12	11	11	11	11

[1]parameters are cumulatively added to each other from top to bottom

[2]conidium death due to slow dew deposition

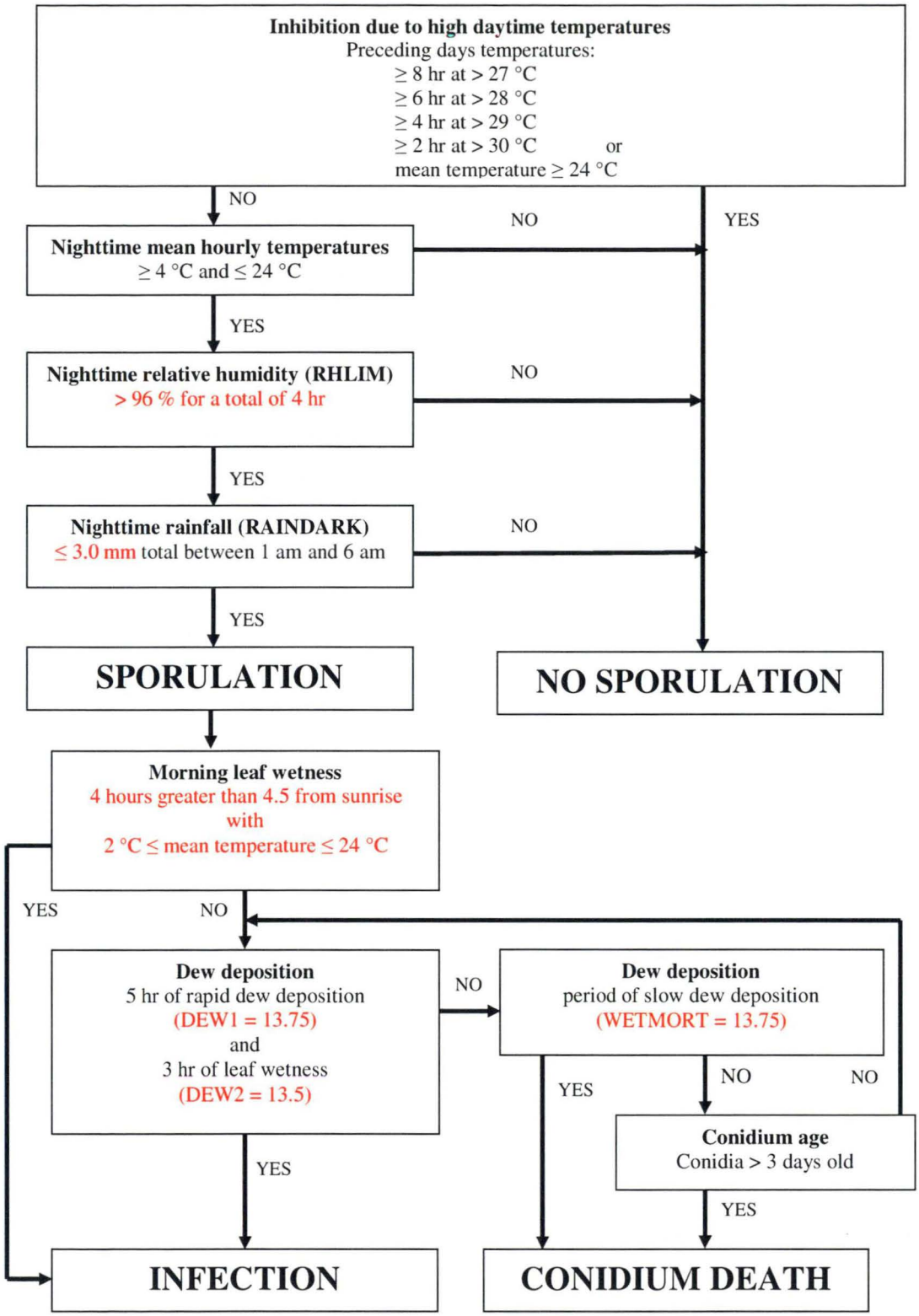


Fig. 5.3 Diagrammatic representation of the forecaster model POPCAST. Changes from onion downy mildew forecaster model, DOWNCAST, are highlighted in red.

5.3.5. Model evaluation

Analysis of weather data collected from crop 1 by the original DOWNCAST model predicted six instances of downy mildew infection subsequent to sporulation, within the crop outside of the active period of applied preventative fungicides (Fig. 5.4 and 5.5). The first of these infection events was predicted at 70 DAS, which would have resulted in the development of symptoms at 77 DAS. In addition, two infection events were predicted at time periods that the crop was protected from infection by fungicide application. The POPCAST model predicted 12 infection events while the crop was unprotected and three while it was protected. The first predicted infection event using the POPCAST model occurred at 55 DAS, which would have resulted in the development of symptoms of infection at 62 DAS. The modDOWNCAST model also predicted the first infection event to occur at 55 DAS. A total of eight unprotected, and two protected, infection events were predicted by the modDOWNCAST model.

Progression of both disease incidence and severity was best described by the logisitic model in crop 1 (Fig. 5.4 and 5.5). During the sampling period, disease incidence increased from 0 at 58 days after sowing (DAS), to 0.89, 86 DAS (Fig. 5.4). During the same time period, disease severity increased from 0 at 58 DAS to 0.08 at 86 DAS (Fig. 5.5).

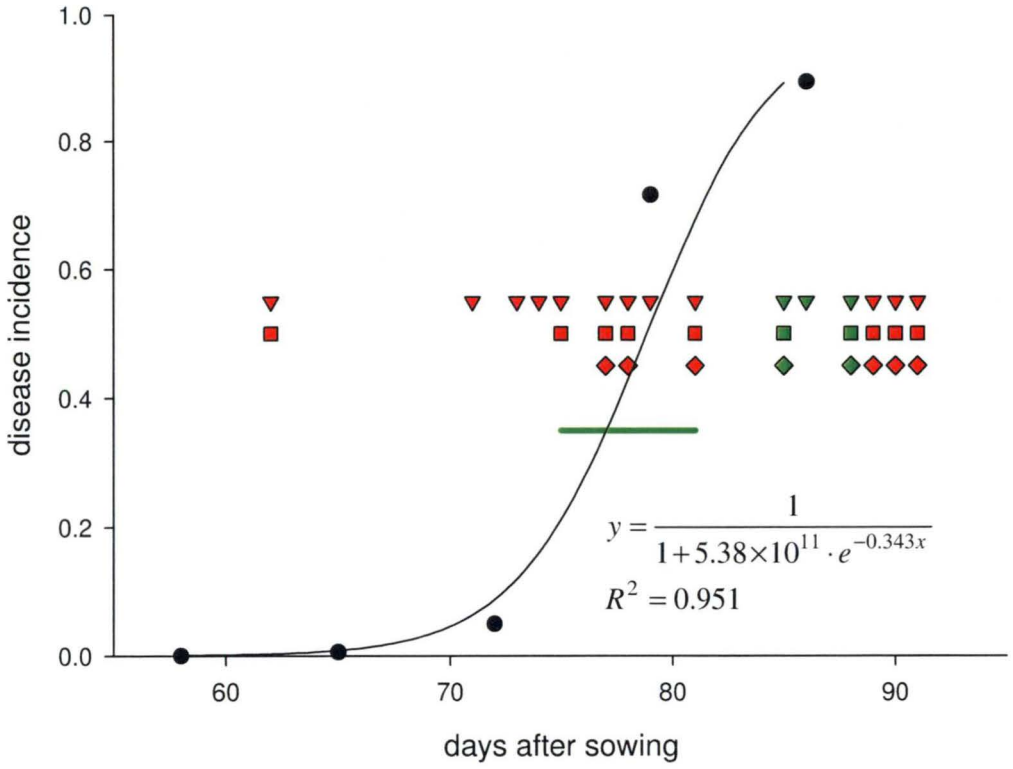


Fig. 5.4 Progression of disease incidence (●) in crop 1, and onset of symptom development based on prediction of infection events by DOWNCAST (♦), modDOWNCAST (■) and POPCAST (▼), assuming a seven day latent period. Green symbols indicate infection events protected against by fungicide application; red symbols indicate uninhibited infection events. Green line indicates the timing of the curative effects of fungicide application.

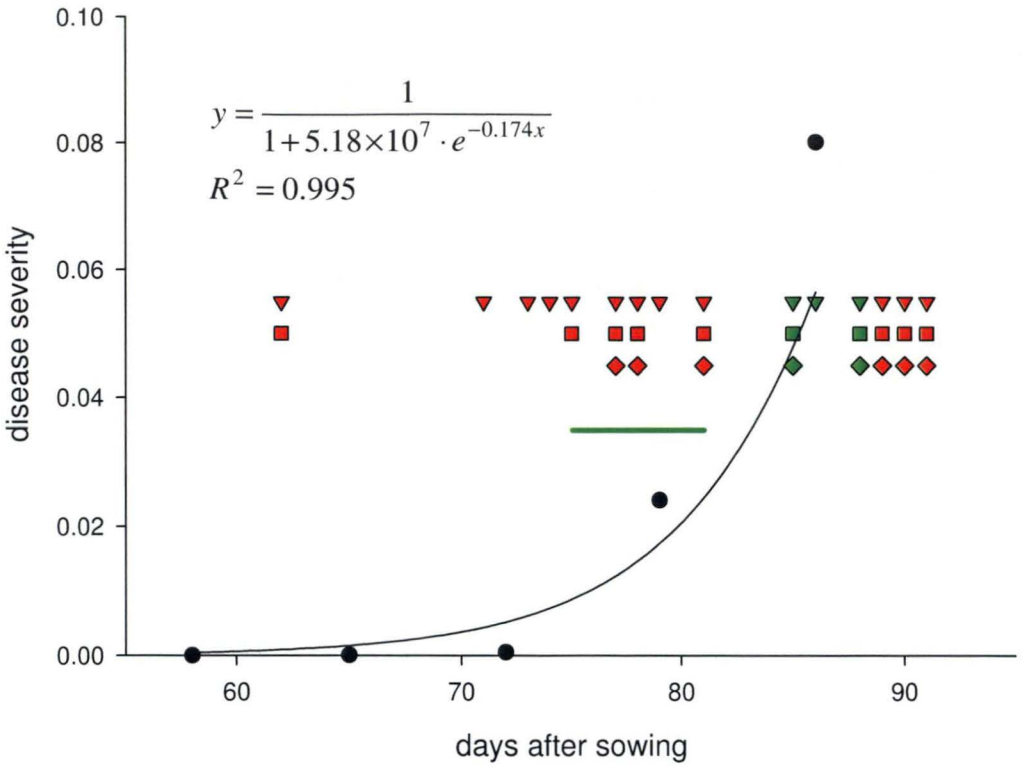


Fig. 5.5 Progression of disease severity (●) in crop 1, and onset of symptom development based on prediction of infection events by DOWNCAST (♦), modDOWNCAST (■) and POPCAST (▼), assuming a seven day latent period. Green symbols indicate infection events protected against by fungicide application; red symbols indicate uninhibited infection events. Green line indicates the timing of the curative effects of fungicide application.

Analysis of weather data collected in crop 2 using all three models predicted the first occurrence of infection at 54 DAS, which, assuming a seven day latent period, would have resulted in the development of symptoms of infection at 61 DAS (Fig. 5.6 and 5.7). The DOWNCAST model predicted the occurrence of 10 unprotected infection events during the monitoring period. In addition, three infection events were predicted to occur during the time period that the crop was protected from infection by fungicides. The POPCAST model predicted the occurrence of 16 unprotected infection events and four protected infection events. The modDOWNCAST model predicted 13 unprotected, and four protected, infection events.

Progression of disease incidence in crop 2 was equally well described by the monomolecular and Gompertz models. The Gompertz model was selected as the model of best fit (Fig. 5.6) as this model inherently describes a dominantly polycyclic disease. During the sampling period, disease incidence increased from 0.068 at 57 DAS to 1.0 from 78 DAS onwards (Fig. 5.6). Progression of disease severity in crop 2 could not be adequately described by any of the standard disease progress curves (Neher *et al.* 1997). Disease severity progress was therefore instead described using a first order quadratic equation, with a predicted peak in disease severity at approximately 75 DAS (Fig. 5.7). Disease severity at crop 2 was 0.00092 at 57 DAS, highest at 71 DAS (0.12), and decreased to 0.071 at 85 DAS.

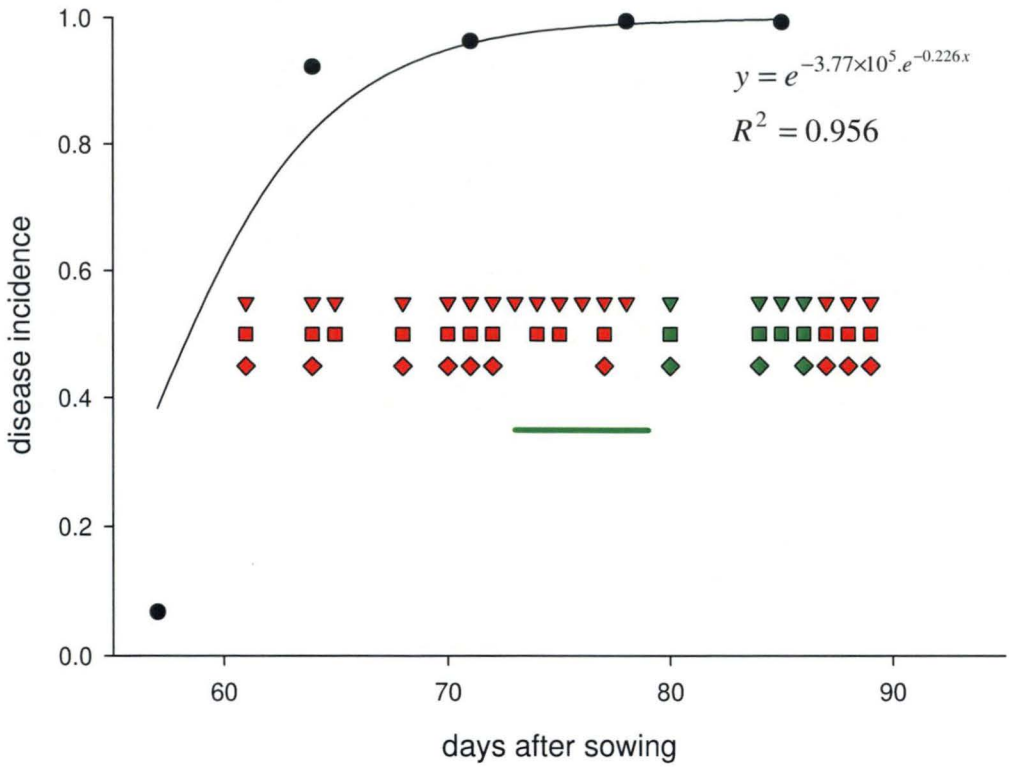


Fig. 5.6 Progression of disease incidence (●) in crop 2, and onset of symptom development based on prediction of infection events by DOWNCAST (♦), modDOWNCAST (■) and POPCAST (▼), assuming a seven day latent period. Green symbols indicate infection events protected against by fungicide application; red symbols indicate uninhibited infection events. Green line indicates the timing of the curative effects of fungicide application.

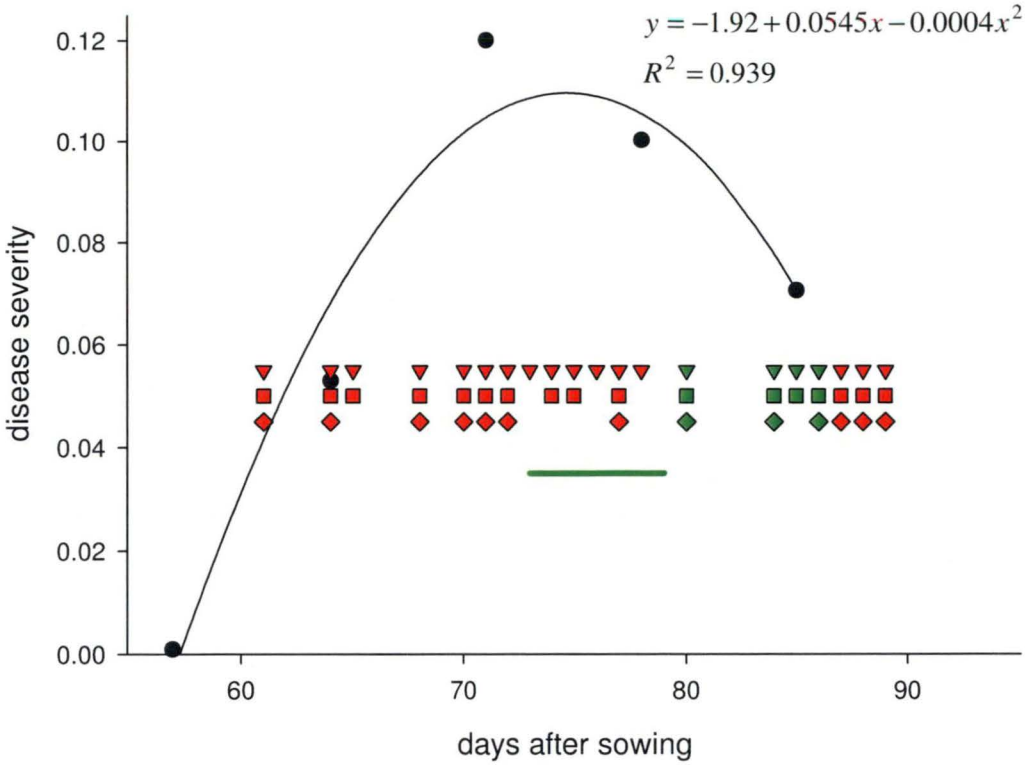


Fig. 5.7 Progression of disease severity (●) in crop 2, and onset of symptom development based on prediction of infection events by DOWNCAST (♦), modDOWNCAST (■) and POPCAST (▼), assuming a seven day latent period. Green symbols indicate infection events protected against by fungicide application; red symbols indicate uninhibited infection events. Green line indicates the timing of the curative effects of fungicide application.

5.4. Discussion

To the author's knowledge, this work constitutes the first attempt to model the interaction between weather variables and the secondary reproduction by *P. cristata*, the causal organism of downy mildew epidemics of oilseed poppy.

Laboratory studies of the effect of temperature on sporulation indicated that *P. cristata* is capable of sporulating at temperatures from 4 to 24 °C. Although temperatures lower than 4 °C were not tested in this study, the very low level of sporulation detected at this temperature (only one sporulating leaf disk producing 33 conidia) would indicate that this temperature is close to the lower limit for sporulation. The range 4 to 24 °C is identical to that incorporated for the prediction of sporulation of onion downy mildew in the model DOWNCAST (Jesperson & Sutton 1987). In addition, evidence collected in laboratory studies indicated that the optimum temperature for the sporulation of *P. cristata* is approximately 16 °C, which is within the optimum range of 12 to 17 °C used by DOWNCAST (de Visser 1998).

Under controlled laboratory conditions, sporulation was not observed at lower than 97 % relative humidity for *P. cristata*. This lower limit for sporulation is higher than the value of HIGHRH (95 % relative humidity) used in DOWNCAST (Jesperson & Sutton 1987). However, similar relative humidity limits for sporulation have been recorded for other *Peronospora* spp., including 97 % relative humidity for *P. trifoliorum* (Fried & Stuteville 1977) and 98 % relative humidity for *P. parasitica* (Paul *et al.* 1998). Results also indicated that the relative humidity requirement for sporulation varied with temperature, with higher relative humidity required to induce sporulation at the temperature extremes relative to the optimum temperatures for

sporulation. However, due to the limited nature of the work conducted here, more studies need to be conducted before adequate conclusions can be drawn as to the nature of this interaction. A similar interaction between temperature and relative humidity is incorporated in DOWNCAST, where the length of time humidity needs to be above the critical limit to allow sporulation to occur is dependent upon mean temperature (de Visser 1998; Jesperson & Sutton 1987). The semi-quantitative prediction of the extent of sporulation by DOWNCAST is influenced by this interaction between temperature and relative humidity. The models ONIMIL (Battilani *et al.* 1996a; Battilani *et al.* 1996b) and the recently described ZWIPERO (Friedrich *et al.* 2003), also scale their quantified prediction of sporulation based on an interaction between temperature and relative humidity.

Analysis of data collected through the monitoring of field sporulation using POPCAST also suggested that increasing the value of RHLIM (the equivalent of HIGHRH) above 95 % improved prediction. The highest levels of accuracy for sporulation prediction were obtained when relative humidity was greater than 96 % for at least 4 hours during the night period, which is in agreement with the laboratory findings that sporulation did not occur at 95 % relative humidity. However, when the interaction between temperature and relative humidity outlined in DOWNCAST was taken in to account, the optimum value of RHLIM was 95 % relative humidity. Some of this discrepancy may be due to the use of trap plants in the field, meaning that conidium deposition had to be used as an indicator of sporulation events. In addition, the requirement of DOWNCAST for the high relative humidity period to end at sunrise, may account for some of the variation between laboratory and field results. ZWIPERO (Friedrich *et al.* 2003) provides an argument for the removal of this requirement, as predictions in this model are based on the accumulation of a

development value equivalent to four hours higher relative humidity under optimum conditions ceasing at any time in the nighttime period. This is analogous with the sporulation prediction sub-unit used in POPCAST.

Use of the initial value of 0.2 mm for RAINLIM in POPCAST predictions was observed to result in several instances of the model failing to predict sporulation events. Increasing RAINLIM to 3.0 mm was observed to significantly improve prediction. The setting of a greater critical limit for the inhibition of sporulation due to rainfall is logical when the relative canopy morphologies of onion and poppy crops are compared. Onions form low, relatively sparse canopies with upright leaf architecture that are very open to rainfall, thus providing little protection to developing conidia from injury. Poppies conversely, form dense, tall canopies over 1.5 m tall, which would effectively provide much greater protection for developing conidia on the abaxial surface of leaves. Additional evidence supporting this alteration is obtained from the ZWIPERO model, which scales the extent of sporulation inhibition, or injury, relative to the level of rainfall, and does not assume total inhibition until a rainfall rate of 2.0 $mm.hr^{-1}$ occurs (Friedrich *et al.* 2003).

Evidence collected in this study suggested that inhibition of sporulation due to high daytime temperatures the preceding day as described by Jesperson and Sutton (1987) and modified by de Visser (1998), improved prediction of sporulation in poppy crops. However, no evidence was obtained in this study for or against the implementation of the rule inhibiting sporulation under extended periods of daytime temperatures greater than, or equal to 34 °C for at least three of the five preceding days, suggested by de Visser (1998). Therefore this rule was not incorporated into POPCAST. However, the rule was maintained in modDOWNCAST, despite having

no effect on prediction. The status of this rule is questionable as de Visser (1998) is the only author to suggest its implementation and in that case it only improved the prediction of DOWNCAST by one sporulation period out of 40.

Reduction of WETINF from the assumed level indicating leaf wetness from 7.5 (based on visual observations) to 4.5 was found to increase the predictive power of POPCAST. de Visser (1998) also found that the critical limit that best predicted infection was lower than the assumed limit for leaf wetness. This suggested that the extent of leaf wetness required by downy mildew conidia is less than is easily detectable by the human eye. If this is the case, this increases the difficulty of calibrating leaf wetness sensors.

The optimum value of WETMORT was found to be equal to DEW1. These results indicate that conidium death occurs if leaf wetness conditions are such that dew formation leading to leaf wetness does occur and initiates infection, but leaf wetness is not maintained long enough to enable infection to be completed. This provides a slightly different aspect to conidium death due to leaf wetness, compared to that of Jesperson and Sutton (1987), who used an ill defined period of slow dew deposition as an indicator of conidium death. It has been shown that slow rates of dew deposition involve cyclic periods of wetting and drying that are assumed to exhaust conidia of their energy supply (Hildebrand & Sutton 1984a). In this work, it is suggested that the energy required to initiate conidium infection depletes the conidium and survival cannot occur if infection is not completed. It is interesting to note that the recently described model, ZWIPERO, does not take into account conidium death due to slow dew deposition rates for onion downy mildew (Friedrich

et al. 2003). Evidence from this study on poppy downy mildew indicates that taking into account slow dew deposition greatly improves prediction.

The original temperature limits of DOWNCAST for infection were found to be too narrow to define infection by poppy downy mildew conidia, with an infection event being recorded at a mean temperature of 2.0 °C. The conidia of the poppy downy mildew pathogen has previously been reported as germinating in the range 2 (Behr 1956) to 26 °C (Yossifovitch 1929). For onion downy mildew, infection has been reported at temperatures as low as 1 °C, and as high as 28 °C (Yarwood 1943). The range 1 to 28 °C is employed by the models ONIMIL (Battilani *et al.* 1996a; Battilani *et al.* 1996b) and ZWIPERO (Friedrich *et al.* 2003). The lower limit for infection was reduced to 2 °C for POPCAST, but not for modDOWNCAST as, based on data collected in this study, prediction was only improved by a single time period. However, from the previously published results above it appears necessary to reduce the lower limit for infection of a modified DOWNCAST to 2 °C and raise the upper limit to 26 °C, or to the same limits employed by ONIMIL and ZWIPERO.

As an intermediate between POPCAST and DOWNCAST, modDOWNCAST was also developed and tested during the course of this work. This model incorporated only those modifications to DOWNCAST used in POPCAST that provided significant improvement to the prediction of DOWNCAST. To this end, modDOWNCAST only incorporated the raise in RAINDARK from 0.2 to 3.0 mm in the sporulation sub-unit. ModDOWNCAST still implemented the interaction between the timing of the onset of relative humidity and the mean temperature of sporulation that is part of DOWNCAST. The difference between predictions based on modDOWNCAST and POPCAST was slight; 84.1 % and 85.7 % respectively

when all other parameters are considered equal and optimum. Therefore it was deemed that the improvement incorporated into DOWNCAST was not significant enough to warrant the removal of the interaction of temperature and relative humidity from the modified version of DOWNCAST, as this was based on extensive research (Jesperson & Sutton 1987), relative to this current study. The infection sub-unit of modDOWNCAST incorporated all of the new leaf wetness parameter values of the POPCAST infection sub-unit. Setting the critical leaf wetness sums for rapid dew formation (SURF1), infection (SURF2) and conidium mortality (MORT) to the values of the equivalent parameters in POPCAST (DEW1, DEW2 and WETMORT respectively) were all shown to improve the prediction of infection relative to the parameters of DOWNCAST.

Evaluation of the three models, DOWNCAST, POPCAST and modDOWNCAST, showed that POPCAST consistently predicted the greatest number of infection events, while DOWNCAST predicted the least number of infection events. All infection events predicted by DOWNCAST were also predicted by both POPCAST and modDOWNCAST, and likewise all infection events predicted by modDOWNCAST were predicted by POPCAST. These results indicate that the three models represent three levels of stringency of prediction. Under highly favourable conditions for epidemic development, such as those seen early in the monitoring period in crop 2 during model evaluation, all three models predict infection events. Under less favourable conditions, infection events were only predicted by POPCAST. In crop 1, disease incidence and disease severity increased at each sampling period relative to the previous sampling period during model evaluation. A single instance of symptom development from a single infection event seven days previous, was predicted by both POPCAST and modDOWNCAST

between 58 and 65 DAS, but not DOWNCAST. Between 65 and 72 DAS, symptom development was only predicted by POPCAST. In subsequent time periods, symptom development was predicted on at least one instance by all three models. In crop 2, where disease incidence rapidly increased to greater than 90 % and was then maintained throughout the sampling period, all three models predicted at least two instances of symptom development between every sampling period. This evidence suggests that under higher favourable weather conditions for disease development all three models would provide adequate prediction of epidemic development. However, under more marginal conditions, such as those that may be observed early season, prior to canopy closure, the DOWNCAST model can fail to predict initial infection events and thus the start of epidemics.

In crop 2, disease incidence increased between 57 and 64 DAS, 64 and 71 DAS, 71 and 78 DAS, but remained steady between 78 and 85 DAS. Disease severity in crop 2 increased between 57 and 64 DAS, 64 and 71 DAS, but then decreased between 71 and 78 DAS, and increased again between 78 and 85 DAS. This decline in disease severity coincides with the application of the fungicides mancozeb and phosphonate. Mancozeb is a non-systemic protectant fungicide, which would prevent the occurrence of further infections, but not prevent disease severity development from infections already present. When the timing of the single mancozeb application is scaled forward seven days to account for the latent period inherent in disease prediction, it coincides with the period between 78 and 85 DAS, where disease incidence did not increase. However it should also be noted that disease incidence at this time was close to 1.0 and little scope for increase existed anyway. Phosphonates are systemic fungicides that are primarily protectant in nature, but do have some curative properties against downy mildews (Guest & Grant 1991; Magarey *et al.*

1991; Magarey *et al.* 1990). The ability of phosphonates to promote the rapid necrosis of existing lesions (Magarey *et al.* 1991; Magarey *et al.* 1990) can therefore be attributed for the decrease in disease severity that coincides with their application. No scaling forward seven days is necessary in this case as the curative effect of the phosphonate would be instantaneous relative to the protective action of the phosphonate and mancozeb combined. The absence of a similar pattern following fungicide application in crop 1 may be due to ineffectual application, although it has been noted previously that the effect of phosphonate application can be variable at times (Magarey *et al.* 1991). The assumed seven day effectiveness of fungicide application is based on industry recommendations for application frequency.

During the course of this study the occurrence of sporulation and infection events under marginal conditions was often variable, making the determination of specific critical limits for prediction, as required by DOWNCAST difficult. Instances of these can be seen from the infection observations starting on the 1st December, 2001 (Appendix VIID). In this case, two plants were observed to be infected on the first day of monitoring, but no plants were infected from those recovered after two and three days monitoring. Three infected plants were observed from those recovered after four days of monitoring. If an infection event had occurred during the first day of monitoring then it would be expected that infected plants would be recovered after every day. Analysis of the weather data collected only predicted infection during the first day of monitoring with WETINF values of 5.5 and lower, suggesting that while conditions allowed infection, they were not highly favourable for such an event. To counter these occurrences a more mathematically based model, such as ZWIPERO, that does not rely as heavily on specific critical limits for infection and sporulation,

but rather scales the level of prediction based on how favourable the conditions are, may be more appropriate.

The results of this study indicate that the forecasting model DOWNCAST, as modified by de Visser (1998), provides a good basis for the prediction of downy mildew epidemics in oilseed poppy crops. Development of the POPCAST model in this work was not designed to develop a new predictive model for use in the control of downy mildew of poppy. POPCAST was instead developed to highlight the key structural and parameter characteristics of DOWNCAST that could potentially be modified to adapt DOWNCAST to the poppy downy mildew pathosystem. The development of DOWNCAST took place over a number of years (Jesperson & Sutton 1987), and since its initial description has continued to evolve (de Visser 1998). On that basis POPCAST cannot be promoted as a true alternative model on the basis of two seasons development. However, several of the parameters of POPCAST did considerably improve prediction, as was seen by the performance of modDOWNCAST. It is therefore felt that DOWNCAST, using the parameters employed by modDOWNCAST, with the alteration of the temperature limits for infection, may provide an accurate prediction of poppy downy mildew epidemics for strategic control. Further validation and development of this model is required, as is investigation of the quantification models ONIMIL and ZWIPERO.

6. Methods of overwintering by the downy mildew pathogen

6.1. Introduction

Obligate pathogens, such as *Peronospora cristata*, are unable to perpetuate in the absence of a compatible host plant species. When the host species in a disease cycle is an annual crop, such as oilseed poppy (*Papaver somniferum*), obligate pathogens require methods of surviving the gap between growing seasons when no living crop host tissue is present. If survival does not occur, no primary inoculum source would exist to initiate epidemics in subsequent seasons. When the host crop is grown during the summer months, and thus absent during the winter period, the survival of the gap period by the pathogen is termed 'overwintering'. In many cases, pathogens may have more than one method of overwintering, and the principal method of overwintering can change between geographic regions (Populer 1981).

Reports vary as to the methods by which the downy mildew pathogen (reported as *Peronospora arborescens*) of oilseed poppy overwinters. In India, overwintering is known to occur through the production of oospores that are reincorporated into the soil with crop residues, providing viable primary inoculum for several seasons (Doshi & Thakore 1991; Doshi & Thakore 2002; Kothari & Prasad 1970; Rathore *et al.* 1987). Inducement of primary infections has also been recorded in Germany through the addition of 'oospore dust', incorporating both oospores and hyphae, to soil (Behr 1956). Reports of the role of seed-borne inoculum are varied. Both Yossifovitch (1929) and Kothari and Prasad (1970) argued that primary infections due to seed-borne inoculum were non-existent. Conversely, Alavi (1975) and Behr (1956) provided circumstantial evidence for seed-borne inoculum. A final alternative for overwintering is through the infection of alternative host species

and/or regrowth *Pap. somniferum* plants that grow during the winter months. Growth of suitable host plants during the winter months provides a 'green bridge' between crop growing seasons enabling the pathogen to survive on living tissue. This method has previously been suggested as a means of overwintering under Tasmanian conditions (Cotterill & Pascoe 1998).

All previous published accounts of poppy downy mildew in Tasmania (Cotterill & Pascoe 1998; Scott *et al.* 2003) have not determined the method, or methods, of overwintering. The aim of this work was, therefore, to evaluate the potential methods of overwintering by the downy mildew pathogen under Tasmanian conditions.

6.2. Materials and methods

6.2.1. Crop residues

Initial examination for survival structures

Leaf samples were collected from commercial crops throughout the north-west region of Tasmania, at the end of the 1999/2000 (1999) growing season. This encompassed the period from the beginning of the maturation of the crop until just prior to harvest. Leaf samples were bulked and 10 random samples were examined for the presence of downy mildew inoculum, by momentarily applying cellotape to the leaf surfaces, placing the cellotape on a glass slide and staining with 0.05 % aniline blue (50 % glycerol, 25 % lactic acid, 25 % H_2O). Slides were examined at 200x magnification using a Zeiss Axiolab compound microscope (Carl Zeiss, Oberkochen, Germany) with Achronplan objective lenses (Carl Zeiss).

Survival and infectivity of oospores over time

Oilseed poppy crop material (leaves and stems) infected with *P. cristata* was collected over the 1999 growing season from commercial crops across the north-west region of Tasmania. The timing of collection varied from January until early May 2000. This encompassed the period from the beginning of the maturation of the crop until after the harvest and just prior to cultivation for the new season's sowing. After collection, samples were bulked and stored under ambient conditions in nylon sacks. In June of 2000, subsamples were encased within 32 bags of fine nylon mesh. Each bag contained 200 to 220 g of dried stem and leaf tissue. The bags were then buried in a field at the TAFE/Freer Farm, Burnie (S41° 04' 25'', E145° 51' 55''; Appendix I) to a depth of 5 to 10 cm. The bags were buried in an uncultivated headland

comprised mainly of grass species. The field was a red ferrosol, Krasnozem soil, with no recent history of poppy cropping to avoid potentially contaminating with oospores of *P. cristata* from previous crops. Bags were recovered from the soil at 0, 1, 3, 7, 13, 19 and 26 months after burial. Four randomly chosen bags were removed at each time interval. At the final sampling, six bags were selected to ensure sufficient material was collected. The material collected from each sampling was then bulked.

Viable spore counts were undertaken initially and subsequent to the recovery of residues, using a modification of the methods outlined by van der Gaag and Frinking (1996a; 1997c). Ten subsamples of 1±0.1 g of residue material were added to 260 mL of sterile distilled water (SDW) and 40 mL of ice, each, and comminuted in a commercial blender for five minutes. Suspensions were then cooled to between 10 and 13 °C in an ice bath, prior to being then sonicated for five minutes in an Ultrasonic cleaner (type Fx8, Unisonics Pty. Ltd., NSW, Australia). Cooling and sonication of the suspension was repeated. The suspension was then poured through nested 50 µm and 20 µm sieves with the contents of the 20 µm sieve washed into a container using SDW and the final volume determined. Where necessary, collected suspensions were concentrated by centrifugation at 2,000 *g* (r_{av} 139 mm) for two minutes in a Harrier 15/80 centrifuge (Sanyo Gallenkamp PLC, England). Following centrifugation, the upper portion of the supernatant was discarded to leave a total volume of approximately 15 mL. The oospore pellet was resuspended in the remaining supernatant through gentle agitation. Suspensions were stored overnight in a refrigerator at approximately 4 °C.

Following storage, 5 mL aliquots were added to equal volumes of 0.01 % 3-(4,5-dimethylthiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT; Merck, Darmstadt, Germany) in 10 mM phosphate buffer (containing a ratio of 3:1 of 10 mM KH_2PO_4 and 10 mM K_2HPO_4, pH 6.4). The resultant solution was incubated for 72 hours at 35 °C. The content of 1 mL of suspension was counted using a plastic counting dish at 100x magnification. Observed oospores were scored based on the colour of their stain; either clear, red, blue (or violet) or black. Red and blue stained oospores were considered viable, while clear and black stained were deemed non-viable (Jiang & Erwin 1990; van der Gaag & Frinking 1997c). An oven dry weight conversion factor was determined by drying three replicates of fresh residue samples of known weight, at 80 °C overnight, weighing to determine the moisture content as a percentage of fresh weight, and determining the mean value. The number of oospores per gram of oven dry weight of tissue was then calculated using the equation:

$$N = \frac{n.v.d}{w.OD}$$

Equation 6.1

where N is the total number of oospores per gram of tissue, n is the number of oospores counted per sample, v is the volume of suspension collected, d is the dilution factor ($d = 2$) to account for the 2x dilution due to the addition of MTT stain, w is the fresh weight of sample and OD is oven dry conversion factor.

The initial infectivity of crop residues and after each recovery was tested by sowing healthy poppy seed into soil-residue mixes. Fifty 20 cm diameter pots were 1/5th filled at the base with potting mix (Appendix V) to aid drainage, and 4/5th filled with a soil-crop residue mixture containing 10 g of crop residues per pot. Soil was a red-

brown clay loam soil (pH 7.0) pasteurised by steam (62 °C for 45 min) prior to use. As a control an additional 50 pots were prepared without the addition of crop residues. Into each pot, four seeds of *Pap. somniferum* were sown. Prior to sowing, seeds were surface sterilised by immersion for five minutes in bleach (0.5 % available chlorine), followed by two rinses in SDW for five minutes each. Pots were then placed under day/night glasshouse conditions of 23±0.1/15±0.1 °C and 73.4±0.4/87.0±0.3 % relative humidity, respectively. Pots were hand watered regularly at the base of plants to prevent leaf wetness and reduce humidity. Seeds were allowed to germinate and develop for one month after sowing, after which germination rate was determined. Seedlings were monitored twice weekly for symptoms of downy mildew infection. Seedlings displaying the symptoms of downy mildew infection were harvested and placed into sealed plastic trays containing moistened tissue paper and incubated at 12 °C and 100 % relative humidity in the dark for 12 hours. Following incubation, the trays were opened and seedlings examined at 65x magnification for the presence of sporulation. Seedlings not displaying symptoms of infection were allowed to develop until the 10 leaf stage, at which point they were harvested and incubated to induce sporulation as described previously.

6.2.2. Seed-borne inoculum

Four seedlots (Table 6.1) of *Pap. somniferum* were tested for the presence of downy mildew inoculum using seed germination, seed washing and molecular detection techniques.

Table 6.1 Description of seedlots tested for the presence of downy mildew inoculum.

Seedlot	Description	Source (date)
T16	Seed collected from untreated plot of a Tasmanian Alkaloids downy mildew fungicide trial.	Tasmanian Alkaloids, Westbury (Aug. 2000)
FT2000	Seed collected from field trial in 2000 growing season (100 % incidence at end of season)[1].	TAFE/Freer Farm, Burnie (Feb. 2001)
13M	Seed lot used to sow field trial in 2001 growing season (0 % initial infection)[1].	Tasmanian Alkaloids, Westbury (Sept. 2001)
GH2002	Seed collected from plants grown in glasshouse and artificially inoculated with downy mildew, prior to flowering.	TIAR glasshouse, Burnie (July 2002)

[1]see Chapter 4

Molecular detection

DNA extractions were undertaken for all seed lots using both a phenol-chloroform protocol and the DNeasy® Plant Mini Kit (QIAGEN, Hilden, Germany.). Both protocols involved the grinding of 100 mg (approx. 200 seeds) of seed in liquid nitrogen with a mortar and pestle, prior to extraction. Three replicates of each seedlot were used for each extraction protocol.

Under the phenol-chloroform protocol, ground seed was added to 500 µL of CTAB buffer (50 mM Tris, pH 8.0, 10 mM EDTA, 700 mM sodium chloride, 1 % (v/v) β-mercaptoethanol, and 1 % (w/v) cetyltrimethylammonium bromide) in a 1.5 mL microcentrifuge tube, vortexed briefly and incubated at 65 °C for one hour. One volume phenol:chloroform:isoamyl alcohol (25:24:1) was added to the lysate, centrifuged at 13,000 *g* (r_{av} 55 mm) for five minutes at room temperature, and the aqueous layer discarded. A second extraction was then undertaken with one volume

of chloroform:isoamyl alcohol (24:1) as previous. DNA was precipitated by adding two volumes of 95 % ethanol and 0.1 vol. of 3 M sodium acetate and incubated at –20 °C for 20 min. DNA was pelleted by centrifuging at 13,000 *g* for five minutes at room temperature, and the supernatant discarded. The DNA pellet was washed with 100 µL of cold 70 % ethanol and suspended in 100 µL SDW.

DNA extraction using the DNeasy kit was conducted according to the manufacturer's specifications. The DNeasy protocol is based on the use of a silica gel membrane to selectively bind DNA in the presence of high salt concentrations, and the elution of DNA from the membrane with low salt solutions. Briefly, following grinding, cell lysis was initiated by adding 400 µL of Buffer AP1 (QIAGEN) and 4 µl of RNase A (100 $mg.mL^{-1}$; QIAGEN)) and incubating for 10 min at 65 °C. Proteins and polysaccharides were then precipitated out of solution by adding 130 µL of Buffer AP2 (QIAGEN), incubating on ice for five minutes and centrifuging at 13,000 *g* (r_{av} 55 mm) for five minutes at room temperature. Precipitates were removed from the lysate by centrifuging through a QIAshredder spin column (QIAGEN) at 13,000 *g* for two minutes at room temperature. DNA was then bound to the silica membrane by adding 1.5 vol. of Buffer AP3/E (QIAGEN), applying to a DNeasy mini spin column and centrifuging at 8,000 *g* (r_{av} 55 mm) for one minute at room temperature, and the flow through discarded. DNA was washed by adding 500 µL of Buffer AW (QIAGEN) to mini spin columns, centrifuging at 8,000 *g* for one minute at room temperature, and the flow through discarded. The wash step was repeated followed by centrifuging at 13,000 *g* for two minutes. Elution of DNA was conducted twice by applying 100 µL of elution buffer AE (QIAGEN), incubating at room temperature for five minutes and centrifuged at 8,000 *g* for one minute at room temperature, to give a final elution volume of 200 µL.

To compare the results of the two extraction protocols, DNA suspensions were separated by gel electrophoresis. Suspensions were added to 1 % agarose gels in 1x TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) stained with ethidium bromide (0.5 $mg.L^{-1}$). Phenol-chloroform extractions were diluted 2x with 1x TAE prior to loading on gels to account for the differing final suspension volumes between protocols. DNA was separated at 100 V for 49 min, and then visualised under UV light.

Extractions obtained by the preferred protocol were then tested for the presence of poppy downy mildew DNA by the polymerase chain reaction (PCR), using the primer pair pdm3/pdm4 (Table 6.2). The amplification mix consisted of 0.1 mM dNTPs, 0.25 µM of each primer, 1x PCR Buffer II (Applied Biosystems, Foster City, CA, USA), 1.5 mM $MgCl_2$, 1 $\mu g.\mu L^{-1}$ bovine serum albumin (BSA), 1 U of AmpliTaq® Polymerase (Applied Biosystems) and 1 µL of DNA extraction, made up to 50 µL with SDW. Amplifications were carried out in a GeneAmp PCR Systems 2400 thermocycler (Perkin Elmer, Norwalk, CT, USA) using an initial denaturation at 94 °C for five minutes followed by 30 cycles of denaturation for 40 s at 92 °C, annealing for two minutes at 53 °C and extension for three minutes at 72°C. This was concluded with a final extension for 10 min at 72 °C. Amplicons were separated in 2 % agarose gels in 1x TAE at 100 V for 49 min, stained with ethidium bromide and visualised under UV light.

Extractions that did not produce amplicons using the primer pair pdm3/pdm4 were tested for quality of the extraction by a second amplification reaction using the generic ribosomal DNA primers ITS1 and ITS4 (Table 6.2; White *et al.* 1990). The

amplification mix used was identical to previous, with the exception of the substitution of primers. Amplifications were carried out using an initial denaturation step of 94 °C for one minute followed by 30 cycles of denaturation for 40 s at 92 °C, annealing for two minutes at 50 °C and extension for three minutes at 72 °C. This was concluded with a final extension for 10 min at 72 °C (Lindqvist *et al.* 1998). Amplicons were separated and visualised as described for the primer pair pdm3/pdm4.

Table 6.2 Primers used for the detection of *Peronospora cristata* inoculum in the seed of *Papaver somniferum.*

Primer	Sense	Sequence (5' → 3')	Location
pdm3[1]	Forward	TCGGTTGGAGCTAGTAGCG	ITS1
pdm4[1]	Reverse	CAACGCCACGCTTTTCA	ITS2
ITS1[2]	Forward	TCCGTAGGTGAACCTGCGG	18S gene
ITS4[2]	Reverse	TCCTCCGCTTATTGATATGC	28S gene

[1]specific to *P. cristata*; see Chapter 3

[2](White *et al.* 1990)

Seed germination

A sample of 0.50 g (approx. 1000 seeds) from each seed lot was sown into seed raising mix (Appendix V). Seeds were then maintained under glasshouse conditions and hand watered at the base of trays at two to three day intervals to prevent the occurrence of high humidity leading to sporulation. Seedlings were maintained until the four leaf growth stage then harvested. Harvested seedlings were incubated at 12 °C and 100 % relative humidity in sealed plastic trays containing moist tissue paper, for between 12 and 48 hours. Individual seedlings were scored for downy mildew infection by microscopic observation of sporulation (65x magnification).

Following visual scoring, 100 seedlings were arbitrarily selected from each seedlot and stained and cleared using a modification of the method of Rumbolz *et al.* (2002). Seedlings were cleared by transferring to 1 M potassium hydroxide and autoclaved for 15 min. Following cooling, seedlings were removed from solution, washed with SDW, and mounted in 0.05 % aniline blue stain (50 % glycerol, 25 % lactic acid, 25 % H_2O). Seedlings were scored visually for the presence of infection structures, at 100x magnification with a Zeiss Axiolab compound microscope with Achronplan objective lenses.

Seed washing

The presence of downy mildew inoculum in seed was also tested using a modification of the seed washing technique of Inaba *et al.* (1983). Ten replicates of 0.50 g (approx. 1000 seeds) of seed from each seed lot were added to 1.5 mL of SDW, and shaken for 10 min. The suspension was pipetted into 1.5 mL microcentrifuge tubes. Solid debris was pelleted by centrifugation at 13,000 *g* (r_{av} 55 mm) for five minutes at room temperature, and the supernatant discarded. The pellet was then resuspended in 50 µL of SDW, and mixed by vortexing briefly.

Suspensions were then visualised at 100x magnification with a Zeiss Axiolab compound with Achronplan objective lenses, by placing 10 µL of suspension on a glass slide and covering with a glass cover slip.

6.2.3. Weeds and alternative hosts

Disease maintenance

Infected poppy plants used in these studies and for downy mildew maintenance were grown as follows. Poppy seed was surface sterilised for five minutes in bleach (0.5 % available chlorine), followed by two washes of five minutes each in SDW. Seed was sown into 20 cm diameter pots containing 4/5th potting mix (Appendix V) and topped with seed raising mix (Appendix V). Pots were maintained under glasshouse conditions, with regular hand watering at the base of plants to prevent the build up of leaf wetness and high relative humidity. Following germination, seedlings were thinned to two per pot. At the 10 leaf growth stage, seedlings had the wax layer of the leaf cuticle removed by rubbing with cotton wool and were misted with a conidium suspension. Conidium suspensions were generated by incubating infected leaves from maintenance poppy plants in sealed containers with damp tissues, at 12 °C and 100 % relative humidity for 12 hours under dark conditions. Conidia were then collected by shaking leaves in distilled water and suspensions were counted with haemocytometer and adjusted to 10^4 to 10^5 conidia.mL^{-1}. Conidium suspensions were applied to plants with a hand mister. Plants were incubated in a moist chamber under glasshouse conditions overnight with regular fine mists, applied for 10 s at five minute intervals during daylight hours, and 30 min intervals under dark conditions, to maintain leaf wetness. Plants were then maintained under glasshouse conditions as described previously.

Weather monitoring

All weather monitoring was conducted using Watchdog® 450 dataloggers (Spectrum Technologies Inc., Plainfield, ILL, USA). Readings were taken for temperature,

relative humidity, rainfall and leaf wetness at 15 min intervals. All readings were taken with sensors placed within plant canopies at 10 cm above ground level. Temperature and relative humidity were recorded using the internal sensors of the Watchdog dataloggers. According to company specifications temperature relative humidity readings had accuracies of ±0.7 °C and ±3 % respectively. Rainfall was monitored with an external, continuous flow through Compact Rainfall Sensors (Spectrum Technologies). Rainfall measurements were recorded in increments of $1/100^{th}$ of an inch, with an accuracy of ±4 %. Leaf wetness was monitored with an external Leaf Wetness Sensor (Spectrum Technologies) sensor. Sensors were flat electrical resistance grids with a range of 0 (dry) to 15 (wet). All weather data collected were analysed using a modified form of the DOWNCAST predictive model, modDOWNCAST, described in Chapter 5 (de Visser 1998; Jesperson & Sutton 1987).

Initial surveys of winter population of regrowth poppies

Surveys for downy mildew on regrowth oilseed poppy population were conducted at the site of the spatiotemporal analysis field trial conducted in the 2000/2001 (2000) growing season at the TAFE/Freer Farm, Burnie (Appendix I and Chapter 4), over the winter months of 2001. Surveys were conducted on the 27^{th} May, 2^{nd} August, 7^{th} September, and 15^{th} October. During each survey all poppy plants present were examined for the presence of downy mildew. Plants exhibiting symptoms of infection were harvested and incubated at 12 °C and 100 % relative humidity in sealed plastic trays containing moist tissues, under dark conditions for 12 hours. Plants were then examined microscopically at 65x magnification for the presence of sporulation. When the observed levels of downy mildew infection were too high for

all plants to be harvested, 30 plants were arbitrarily selected from the poppy population for incubation.

Pot trials in 2002

The ability of downy mildew to survive on host plants was monitored in the winter of 2002. Fifty artificially inoculated plants, as described previously for disease maintenance, were placed in an external environment outside the glasshouse of the North-West Centre of the University of Tasmania, Burnie (S41° 04', E145 52'; Appendix I). Plants were maintained under ambient conditions for the winter period from the 21st May to the 11th September (WINTER) with weather conditions monitored throughout that period. At the end of the exposure period, all plants were harvested and incubated for 12 hours under darkness at 12 °C and 100 % relative humidity in sealed containers with moist tissue. Following incubation, all plants were examined microscopically at 65x magnification for the presence of sporulation. In addition, 50 plants were placed in a separate position at the same site at monthly intervals with a second weather station to monitor conditions. In these instances, plants were placed on the 21st May (MAY), 18th June (JUNE), 18th July (JULY), and the 14th August (AUGUST) and harvested on the 18th June, 18th July, 14th August and the 11th September, respectively. Following harvest, plants were treated as previously described.

The occurrence of environmental conditions suitable for sporulation, separate from infection, was also monitored during the winter of 2002 using potted plants. Fifty plants at the 10 leaf growth stage, were infected with downy mildew as discussed previously and maintained under dry glasshouse conditions of day/night, ±standard

error of mean 22±0.1/14±0.1 °C, and 64±0.6/79±0.4 % relative humidity, respectively, for 10 days. These were placed in an external environment situated at the North-West Centre on the 2nd June, 2002, with weather monitored as described previously. Plants were maintained at ambient conditions for 10 days. At daily intervals four healthy seedlings (at 10 leaf stage) were placed in close proximity to the infected seedlings and removed the following day. After returning to the glasshouse, trap plants were incubated within a moist chamber under glasshouse conditions overnight with the same misting regime as per downy mildew maintenance, to induce infection of trap plants by conidia present. Following incubation, plants were maintained in a dry glasshouse and hand watered at the pot base for 14 days. Plants were then harvested and incubated at 12 °C and 100 % relative humidity in sealed containers with moist tissue for 12 hours and then examined at 65x magnification for the presence of sporulation. A single plant exhibiting sporulation was considered an indication of a sporulation event for that trapping day. For all days, an additional two plants were maintained under dry glasshouse conditions as a control.

Alternative hosts

The ability of related plants species to act as alternative hosts for downy mildew was tested by a modified form of Koch's postulates accounting for the obligate nature of downy mildew of poppy. Poppy species tested were those common to Tasmania. These included the common weeds of cultivation *Papaver argemone*, *Pap. dubium*, *Pap. hybridum*, *Pap. rhoeas*, and *Pap. setigerum*, the ornamental species *Pap. nudicaule*, *Pap. orientale* and *Eschcholtzia californica*, and the legally controlled species *Pap. bracteatum*. Annual poppy species (*Pap. argemone, Pap. dubium, Pap.*

hybridum, Pap. rhoeas, Pap. setigerum, Pap. nudicaule and *E. californica*) were sown as outlined for host maintenance and grown until the small rosette stage of development prior to testing. The perennial species (*Pap. bracteatum* and *Pap. orientale*) were collected as rootstocks from sites around Westbury (Appendix I), planted in potting mix (Appendix V) and maintained until fresh leaf growth developed prior to testing.

Potential host species were tested by misting with approximately 2 mL of conidium suspension prepared as described for disease maintenance previously, and incubated in a moist chamber as for disease maintenance. Plants were then maintained for 14 days under dry glasshouse conditions. Plants were examined for the presence of mildew symptoms, harvested and incubated at 12 °C and 100 % relative humidity in sealed containers with moist tissues, in the dark for 12 hours. Plants were observed microscopically at 65x magnification for the presence of sporulation. The sporulating leaves of individual species were bulked and used to generate fresh conidium suspensions (as previous), which were then used to inoculate four healthy seedlings of *Pap. somniferum* at the 10 leaf growth stage. Inoculated seedlings were then incubated to induce infection as previous, and maintained for 14 days under dry glasshouse conditions. *Papaver somniferum* seedlings were then harvested, incubated to induce sporulation, and examined microscopically. Potential host species that exhibited sporulation, and produced conidia that were able to infect healthy *Pap. somniferum* plants were deemed hosts of the downy mildew pathogen. The number of plants tested for each species varied in this study and was dependent upon the availability of plants. For all instances of inoculation and incubation to promote infection, four healthy seedlings of *Pap. somniferum* were also inoculated and incubated as controls to confirm favourable conditions for infection.

6.3. Results

6.3.1. Crop residues

Initial examination for survival structures

Initial microscopic observations of the surface of downy mildew infected leaves obtained from commercial crops towards the end of the crop life, revealed the presence of oospores (Fig. 6.1), 25 to 40 µm in diameter, on both dried and green leaf tissues. On dried leaf tissue only oospores were observed (Fig. 6.1A). On green leaf tissue both conidia and oospores were detected (Fig. 6.1B).

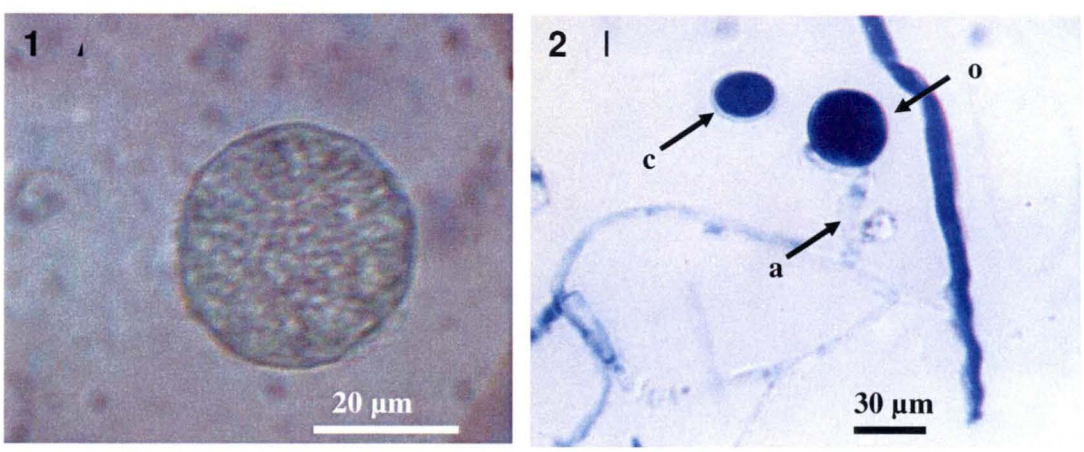


Fig. 6.1 Oospores extracted from the surface of a downy mildew infected leaf of *Papaver somniferum*. A) oospore unstained; B) oospore (o) with antheridium (a) still attached and conidium (c) in background, stained with 0.05 % aniline blue.

Survival and infectivity of oospores over time

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The initial density of oospores was 11,000 oospores per oven dry gram of plant tissue (OPG), of which 1,300 OPG were stained either red or blue (Fig. 6.2), indicating 11 % viability. Over time the total number of oospores declined to a minimum of 2,100 OPG after 13 months burial. At the same point in time the number of viable oospores had decreased to 880 OPG, or 42 % of all oospores. Oospores were still

detected 26 months after burial, with the total number of oospores being 2,900 OPG, and the viable oospores being 1,400 OPG (47 % viability).

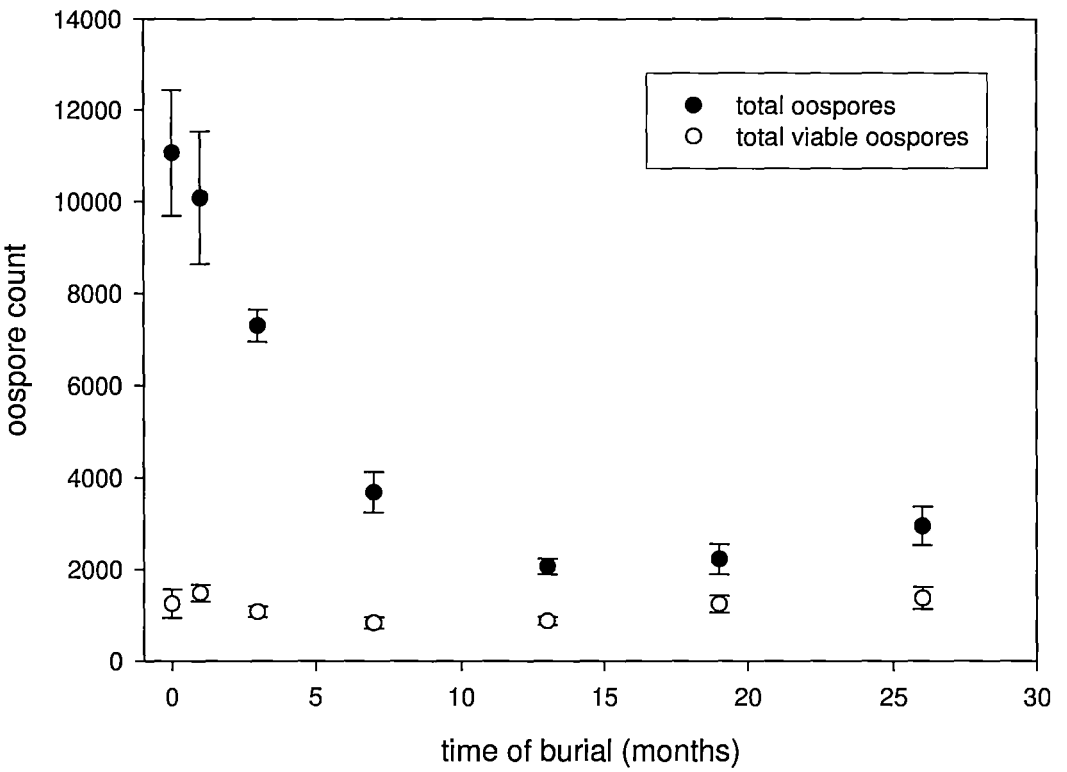

Fig. 6.2 Temporal changes in numbers of oospores extracted from crop residues of *Papaver somniferum* during burial. Bars represent the standard error of the mean.

No downy mildew infections were detected in any seedlings sown either in soil, or soil-residue mix at any time period (Table 6.3). Seedling germination levels varied from 75 % of seed sown in soil-residue mix containing residue buried for 19 months to 15 % of seed sown in residue buried for 26 months.

Table 6.3 Number of germinating seedlings from surface sterilised seed and the number of seedlings infected by downy mildew, sown into mixtures of soil and oilseed poppy crop residues buried within nylon bags for varying lengths of time.

Burial time (months)	# germinating seedlings[1]	# infected seedlings
0	102	0
1	68	0
3	55	0
7	55	0
13	112	0
19	153	0
26	30	0

[1]out of a total of 200 seeds sown

6.3.2. Seed-borne inoculum

Molecular detection

Levels of DNA detectable by agarose gel electrophoresis were recovered from both the phenol:chloroform and DNeasy® Plant Mini Kit (QIAGEN) seed extractions (Fig. 6.3). DNA extractions using the DNeasy protocol produced single, high molecular weight bands that equated to between 4 and 8 $ng.\mu L^{-1}$ in concentration (Fig. 6.3). Phenol-chloroform extractions produced high molecular weight bands, less than 4 $ng.\mu L^{-1}$ in concentration, that were accompanied by strong smears of DNA less than 500 base pairs (bp) in size (Fig. 6.3).

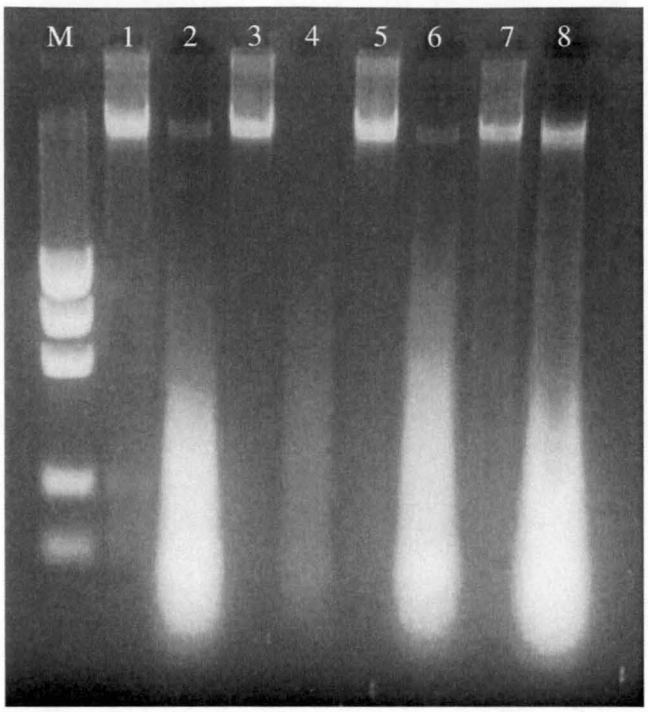

Fig. 6.3 Agarose gel of DNA extractions from seed. Lane M: molecular mass standard with bands equal to 1000, 700, 500, 200 and 100 base pairs respectively, strength of bands equates to 40, 28, 20, 8 and 4 $ng.\mu L^{-1}$ respectively; lanes 1-2: seedlot FT2000; lanes 3-4: seedlot T16; lanes 5-6: seedlot 13M, lanes 7-8: seedlot GH2002; lanes 1, 3, 5 and 7: DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) extractions; lanes 2, 4, 6 and 8: phenol-chloroform extractions.

All replicate extractions of each seedlot tested with the *P. cristata* specific primer pair, pdm3/pdm4, reacted to produce bands approximately 390 bp in size (Fig. 6.4). Most extractions produced weak reactions only. Replicate extraction two from seedlot T16 produced the strongest reaction product.

As all extractions tested positive using the primer pair pdm3/pdm4, no testing with the primers ITS1 and ITS4 was necessary in this study.

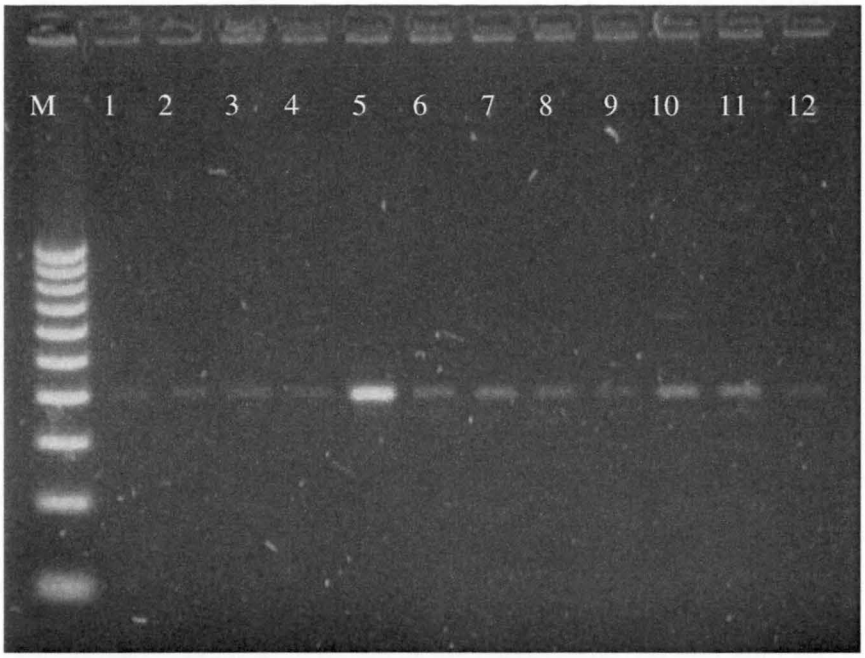

Fig. 6.4 Amplicons derived from DNA extractions from oilseed poppy seed with *Peronospora cristata* specific primer pair pdm3/pdm4. Lane M: 100 base pair (bp) increment ruler with lowermost band equal to 100 bp; lanes 1-3: seedlot FT2000; lanes 4-6: seedlot T16; lanes 7-9: seedlot 13M; lanes 10-12: seedlot GH2002.

Seed germination

The number of seedlings developing out of 1000 seed sown varied between 124 for seedlot GH2002, and 818 for seedlot 13M (Table 6.4). No seedlings from any seedlot were observed to exhibit sporulation following incubation under conditions suitable for sporulation (Table 6.4). Examination of seedlings following clearing and staining did not show the presence of any infection structures within any of the seedlings of any seedlot (Table 6.4).

Table 6.4 Detection of *Peronospora cristata* in germinated oilseed poppy seedlings by incubation, or clearing and staining.

Seedlot	# seedlings[1]	# sporulating seedlings[2]	# infected seedlings[3]
T16	658	0	0
FT2000	758	0	0
13M	818	0	0
GH2002	124	0	0

[1]number of seedlings developing out of 1000 seeds sown

[2]number of seedlings displaying sporulation of *P. cristata* after incubation under favourable conditions

[3]number of seedlings (out of 100) found infected by *P. cristata* following clearing and staining with aniline blue

Seed washing

Oospores were detected in all seedlots following seed washing (Table 6.5). The highest concentration of oospores was detected in seedlot GH2002, with a mean of 1544 oospores.g^{-1} of seed. For other seedlots, the mean concentration varied between 13 (seedlot 13M) and 33 (seedlot FT2000) oospores.g^{-1} of seed. Neither conidia, nor mycelial fragments, were detected in any seedlot.

Table 6.5 Observed number of oospores collected by seed washing from different seedlots of *Papaver somniferum.*

Seedlot	Oospores ($.g^{-1}$ seed)[1]	SEM[2]
T16	16	5.42
FT2000	33	12.0
13M	13	4.73
GH2002	1544	225

[1]mean number of oospores detected from ten replicates of 0.5 g of oilseed poppy seed

[2]standard error of mean

6.3.3. Weeds and alternative hosts

Initial surveys of winter population of regrowth poppies

On the 27th May, 100 days after harvest, downy mildew incidence was observed to be high in regrowth poppies in the field, with 23 of the 30 arbitrarily sampled seedlings sporulating under favourable conditions (Table 6.6). On the 2nd August downy mildew incidence was much lower with only two infected seedlings detected. No downy mildew infections were detected on the 7th September or the 29th October. Viable host tissue was observed at each sampling period.

Table 6.6 Observations of downy mildew incidence in regrowth *Papaver somniferum* population during winter of 2001 at TAFE/Freer Farm, Burnie.

Survey date	Observations
27 May	High incidence of downy mildew symptoms in field 30 seedlings randomly selected for incubation to promote sporulation Sporulation observed from 23 seedlings
2 August	Low incidence of downy mildew symptoms in field 23 suspected infected plants sampled for incubation to promote sporulation Sporulation observed from two seedlings
7 September	Low incidence of downy mildew symptoms in field 20 suspected infected plants sampled for incubation to promote sporulation No sporulation observed
29 October	No downy mildew symptoms observed in field 30 randomly sampled plants sampled for incubation to promote sporulation to check for latent infections No sporulation observed

Pot trials in winter 2002

Of the 50 initial seedlings artificially inoculated with downy mildew and placed outside, 40 plants survived the WINTER exposure period (Table 6.7). Of these, 26 plants were still infected by downy mildew at the end of the exposure period. All 50 plants survived the MAY exposure period, and all plants sporulated following incubation at the end of the period. Nine of the 10 plants that survived the JUNE exposure period were infected with downy mildew at the end of the period. No plants survived the JULY exposure period. Eleven plants survived the AUGUST exposure period, with two of those plants infected by downy mildew at the end of the period.

Analysis of weather data collected (Appendices VIIIA to VIIIE) using modDOWNCAST (see Chapter 5) predicted a total of 44 infection events over the WINTER period (Table 6.7). Analysis of weather data collected over single month periods, led to 10, 11, 9 and 6 predicted infection events in the periods MAY, JUNE, JULY and AUGUST respectively.

Table 6.7 Survival of downy mildew epidemics during the winter months of 2002.

Monitoring period	**# surviving plants**	**# infected plants**	**# predicted infection events[1]**
21 May to 18 June (MAY)	50	50	10
18 June to 18 July (JUNE)	10	9	11
18 July to 14 August (JULY)	0[2]	0	9
14 August to 11 September (AUGUST)	11	2	6
21 May to 11 September (WINTER)	40	26	44

[1]number of prediction infection events based on analysis of weather data collected by modified form of the predictive model, DOWNCAST; see Chapter 5

[2]all plants died during this monitoring period due to damage from climatic conditions and vertebrate pests

Trap plants were infected by downy mildew on every day of trapping under ambient winter conditions (Table 6.8). All four trap plants were infected on the 3rd, 4th, 5th, 6th, 7th 8th and 10th of June. On the 9th, 11th and 12th of June three out of the four trap plants were infected. Analysis of weather data collected (Appendix VIIIF) by modDOWNCAST predicted the occurrence of sporulation on the 3rd, 4th, 5th, 6th, 8th and 9th of June.

Table 6.8 Observed and predicted incidence of sporulation of *Peronspora cristata* from artificially inoculated *Papaver somniferum* plants, exposed to ambient conditions.

Date	# infected seedlings[1]	Sporulation prediction[2]
3 June 2002	4	1
4 June 2002	4	1
5 June 2002	4	1
6 June 2002	4	1
7 June 2002	4	0
8 June 2002	4	1
9 June 2002	3	1
10 June 2002	4	0
11 June 2002	3	0
12 June 2002	3	0

[1]out of four trap plants

[2]values of 1 indicate predicted sporulation, while 0 indicates no sporulation; predictions based on the analysis of weather data by a modified form of the predictive model DOWNCAST; see Chapter 5

Alternative hosts

Artificial inoculation of the species *Pap. bracteatum*, *Pap. dubium*, *Pap. orientale*, *Pap. rhoeas* and *Pap. setigerum* all resulted in downy mildew-like symptoms (Table 6.8). Incubation of these species to promote the occurrence of sporulation led to the production of conidiophores and conidia with the same morphology as *P. cristata.* Inoculation of healthy *Pap. somniferum* seedlings with conidia from each of these species led to typical symptoms of downy mildew infection. Typical downy mildew sporulation was observed when these *Pap. somniferum* plants were incubated to promote sporulation.

The species *E. californica, Pap. argemone, Pap. hybridum and Pap. nudicaule* did not exhibit the symptoms of downy mildew infection, nor produced downy mildew conidia following artificial inoculation (Table 6.9).

Table 6.9 Potential host species of *Peronospora cristata* and results of challenge tests.

Species	**# plants tested**	**# infected plants**	**# infected *Pap. somniferum* plants[1]**
Eschcholtzia californica	82	0	-[2]
Papaver argemone	9	0	-
Pap. bracteatum	24	3	3
Pap. dubium	17	5	3
Pap. hybridum	99	0	-
Pap. nudicaule	133	0	-
Pap. orientale	26	7	4
Pap. rhoeas	15	4	4
Pap. setigerum	7	7	4

[1]number of infected *Pap. somniferum* plants (out of four) infected by conidia produced on the leaves of the individual potential host species to complete Koch's postulates

[2]- indicates that no *Pap. somniferum* plants were inoculated as no infection of the potential host occurred

6.4. Discussion

Analysis of leaves collected from poppy plants infected with *P. cristata* revealed the presence of oospores typical of the genus *Peronospora*. This is the first record of oospores being associated with downy mildew infections of poppy in Tasmania. Oospores were measured at 25 to 40 µm in diameter, which generally agreed with the measurements reported for the oospores of *P. arborescens* on poppy of Yossifovitch (23 to 39 µm; 1929), Behr (mean 30.4 µm; 1956) and Scharif (35 to 54 µm; 1970), but were larger than those of Maiti and Chattopadhyay (22 to 23 µm; 1986) and smaller than those of Francis (42 to 48 µm; 1981). This highlights the large variation in size that can occur with different climatic and geographic conditions, or possibly the differences between *P. cristata* and *P. arborescens*. Whether or not the relative size of oospores is related to the relative infectivity of oospores is subject to conjecture and requires further study.

Over the course of a 26 month burial period the number of oospores extracted from crop residues reduced from an initial 11,100 OPG to a minimum of 2,100 OPG at 13 months burial. After 13 months, the level of oospores extracted from recovered material increased slightly to 2,900 OPG at 26 months burial. This apparent increase in oospores may be an artefact of this study created by a faster rate of decomposition of buried crop residues relative to the rate of oospore decomposition. Unfortunately, the total weights of material recovered at each time period was not recorded, and as such the total number of oospores remaining in recovered samples cannot be calculated. Over the course of the burial period the number of recovered viable conidia did not significantly change. This suggests that the rate of oospore death was proportional to the rate of residue decomposition. Of the initial 11,100 OPG only

11.3 % were detected as viable. This agrees with the findings of van der Gaag and Frinking (1997b), who found that for *P. viciae* f.sp. *pisi*, only a small proportion of the initial oospore inoculum were able to form the basis of primary inoculum the following season. In my study, viable inoculum was still detected 26 months after burial. Converse to these results, however, was the failure to induce infection of poppy seedlings by the addition of oospore infested residues to the soil of germinating seedlings. This absence of infection questions the infectivity of oospores under Tasmanian conditions. Further work is required to assess the ability of oospores collected from Tasmanian poppy crops to germinate and subsequently infect poppy seedlings. Previous studies have reported difficulty in the inducement of the germination of oospores under laboratory conditions (Behr 1956; Kothari & Prasad 1970).

DNA extraction using the DNeasy protocol produced higher quantities of higher quality DNA from seed relative to the phenol-chloroform protocol. Although overall amounts of DNA recovered were similar for both protocols, less DNA shearing was detected using the standard DNeasy protocol. In addition, extraction via the DNeasy protocol was found to be more rapid. For these reasons, DNA recovered via the DNeasy protocol was preferred to that from the phenol-chloroform protocol when it came to testing extractions for the presence of *P. cristata* DNA. Aegerter *et al.* (2002) also reported that extraction of DNA using a modified form of the DNeasy protocol was more effective than a phenol-chloroform based method for the extraction of *P. sparsa* from rose rootstock samples. PCR amplification of DNA extractions from seed with specific primer pair pdm3/pdm4 indicated the presence of *P. cristata* DNA in all samples tested. This test shows that *P. cristata* inoculum is

associated with poppy seed, however it does not indicate whether this inoculum was viable, or its location in the seed.

No symptoms or signs of infection were detected in germinated seedlings from any seedlot, using either seedling incubation to induce sporulation, or clearing and staining, as the means of detection. Using seed washing, however, oospores typical of poppy downy mildew were detected in all seedlots. For the seedlots 13M, T16 and FT2000 the number of oospores detected were very low. The oospores of *Peronospora* spp. are commonly associated with the seed coats of soybean (Jones & Torrie 1946; Pathak *et al.* 1978; Roongruangsree *et al.* 1988), pea (Mence & Pegg 1971), buckwheat (Zimmer *et al.* 1992), spinach (Cook 1935) and brassica species (Vishunavat & Kolte 1993). In addition, oospores have been found internal to the seed coat of buckwheat (Zimmer *et al.* 1992) and soybean (Pathak *et al.* 1978), while mycelia has been detected inside the seed coat of soybean (Roongruangsree *et al.* 1988), and brassica species (Achar 1995; Jang & Safeeulla 1990b). It is therefore possible that internal inoculum is also present in the seeds of poppy. The absence of symptoms of infection despite the detection of inoculum by both seed washing and molecular testing suggests that no transmission occurs, or the transmission rate of the inoculum present was lower than was detectable in the current study. Low primary infection rates (0.4 to 0.9 %) have previously been recorded for brassica species infested with the oospores of *P. parasitica* (Vishunavat & Kolte 1993). The seedlot GH2002 was infested with significantly higher levels of oospores than all other seedlots tested in this study. This seedlot was also observed to germinate at much lower rates than all other seedlots, however, it was not possible to test the significance of this relationship due to the absence of repetition in the seed

germination data. The possibility that high levels of oospore infestation significantly decrease seed germination rates requires further work.

Surveys during the winter and early spring of 2001 of the headlands of a field in which a poppy crop had been grown the previous season, revealed the presence of regrowth oilseed poppy plants throughout this time. Observations suggested that these plants were from continual germination of seeds during the autumn and winter periods. Within that population of host plants downy mildew infected individuals were detected on the 27th May and the 2nd August, but not on the 7th September or the 29th October. The absence of downy mildew infection in these later two time periods, despite the presence of available host plants suggests several possibilities. Firstly, host plants infected with downy mildew died out during the colder period of winter without transmission to seedlings germinating in late winter. Secondly, downy mildew infections were eradicated from infected plants during the colder period of winter. Finally, weather conditions during the winter months in Tasmania may not be favourable for secondary infection events. Monitoring of artificially inoculated poppy plants during the winter of 2002 found that downy mildew infections were maintained throughout the winter period and each individual month where poppy plants survived. Failure of poppy plants to survive the JULY monitoring period was principally due to browsing damage by vertebrate pests, with frost conditions finishing off damaged seedlings. Analysis of weather conditions with a modified form of the forecasting model, DOWNCAST, predicted infection periods throughout the monitoring period. The number of predicted infection periods varied between six in the AUGUST period and 11 in the JUNE period, with a total of 44 infection periods predicted over the entire WINTER period. In addition, monitoring of sporulation events during June led to six events both predicted and

observed. An additional four sporulation events observed may have been due to the presence of residual conidia from previous sporulation events and the close proximity between infected hosts and trap plants under the artificial situation of potted plants. All these results indicate that winter conditions were favourable for the secondary transmission of downy mildew. The absence of downy mildew infection from the later two survey periods of winter 2001, is therefore likely to be due to the death of host plants without transmission to freshly germinating seedlings, in that instance.

In addition to *Pap. somniferum*, the species *Pap. bracteatum, Pap. dubium, Pap. orientale, Pap. rhoeas and Pap. setigerum* were confirmed in this study as viable hosts of *P. cristata*. *Papaver bracteatum* has not been previously recorded as susceptible to infection by either *P. cristata* or *P. arborescens*. No evidence was obtained to indicate the species *Eschcholtzia californica*, *Pap. argemone, Pap. hybridum* or *Pap. nudicaule* are viable hosts of *P. cristata.* This contradicts with previous work that has shown that *Pap. nudicaule* is susceptible to infection by *P. arborescens* (Alcock 1933) and *Pap. hybridum* to *P. cristata* (Constantinescu 1991). These discrepancies may be explained by the confusion between *P. arborescens* and *P. cristata* (Chapter 3). However, *Pap. argemone* has been recorded as a host of both *P. arborescens* (Cotton 1929) and *P. cristata* (Gaumann 1923; Gustavsson 1959), despite not being infected by *P. cristata* during the course of this study. This may be due to pathotype differences, although it is acknowledged that only limited testing of *Pap. argemone* was carried out in this work due to poor germination rates of the seed used. This work confirmed that ornamental species such as *Pap. rhoeas* and *Pap. orientale* that are commonly found in Tasmanian gardens, and common weed species including *Pap. dubium*, *Pap. rhoeas*, and

Pap. setigerum, can act as a green bridge for the overwintering of *P. cristata* in the absence of oilseed poppy crops.

In summary, this work has shown that the sexual spore of *P. cristata* is present both in the residues of host material and in seed, but no evidence of primary infection from either source was obtained. It is possible that the rate of infection by oospores is lower than the detection limits employed in this work. The most likely method of overwintering by *P. cristata* appears to be on the range of poppy species, including regrowth *Pap. somniferum*, that are present during the winter months in Tasmania. During winter of 2002 at least, conditions were conducive to the continuation of asexual reproduction. Further study is required to assess whether or not the conditions recorded in 2002 were typical of the Tasmanian winter climate. However, if historical data is to be used in such an analysis, a reliable source of leaf wetness readings must be obtained.

7. General discussion and suggestions for further research

7.1. General discussion

Analysis of the ribosomal DNA (rDNA) region, including the internal transcribed spacer regions (ITS1, and ITS2), and the 5.8S gene, indicated that two *Peronospora* species are able to infect *Papaver* spp., *Peronospora cristata* and *P. arborescens*. In addition, it was indicated that of the downy mildew pathogen of oilseed poppy is *P. cristata*, not *P. arborescens* as previously reported (Cotterill & Pascoe 1998). While the morphological dimensions, the traditional method of differentiation between *P. arborescens* and *P. cristata*, of poppy downy mildew collections from Tasmania were inconclusive, collections had greater sequence homologies over the ITS region with *P. cristata* (99 %) relative to *P. arborescens* (92 %). Phylogenetic analysis using both distance and maximum parsimony methods consistently indicated that *P. arborescens* was more distantly related to Tasmanian poppy downy mildew collections than *P. cristata*, as well as *P. destructor, P. rumicis* and *P. farinosa*. The establishment of *P. cristata* as the pathogen of oilseed poppy in Tasmania, raises the question of the pathogenicity of *P. arborescens* to oilseed poppy. To the author's knowledge, no other instances of molecular analysis being used to determine the species of *Peronospora* infecting oilseed poppy have been reported.

Monitoring the spatiotemporal dynamics of poppy downy mildew epidemics demonstrated that, under favourable conditions, epidemics are able to develop rapidly from very low rates of primary infection. The development of epidemics was characteristic of predominantly polycyclic diseases, with the progression of disease incidence and severity characterised by the logistic and exponential models respectively. The spatial pattern of epidemics was aggregated after the onset of

canopy closure, and the spatial pattern at one time period was closely associated to the spatial pattern of 10 days previous. These results indicate localised secondary spread dominates epidemic development. In addition, under non-random spatial patterns of plant density, both disease incidence and severity were both correlated and spatially associated with plant density after the onset of canopy closure. These results indicate that the change in crop microclimate, especially higher relative humidities, after canopy closure specifically favour secondary spread. High nighttime relative humidity has been shown to favour sporulation events, facilitating secondary spread.

The effect of downy mildew infection on the yield of poppy crops was principally through the reduction of alkaloid content of capsule dry matter. Alkaloid content was negatively correlated and spatially dissociated with disease incidence and/or severity at many time periods post-canopy closure during both the 2000/2001 and 2001/2002 growing seasons. No instances of a positive relationship between disease incidence or severity and alkaloid content were recorded. The relationship between capsule dry matter yield and downy mildew was less clear, principally because disease incidence and severity was favoured by higher local plant densities, which also favoured increased dry matter yield. This masked any negative effect on dry matter yield that downy mildew infection may have.

The onion downy mildew forecaster model, DOWNCAST, was found to provide moderate prediction of poppy downy mildew epidemics. Further improvement to prediction was obtained by increasing the critical limit for nighttime rainfall inhibition of sporulation from 0.2 to 3.0 mm, and reducing the critical leaf wetness reading (using Watchdog™ 450 dataloggers) required for the infection process, from

7.5 to 4.5. It can therefore be concluded that the key requirements of the sporulation process of poppy downy mildew are at least four hours of relative humidity greater than 95 % during an individual nighttime period, with rainfall less than or up to 3.0 mm. The key requirements for infection of host tissue by the conidia formed by sporulation are a period of rapid dew formation, followed by a three hour period of sustained leaf wetness. Conidium death occurs if rapid dew formation occurs, but leaf wetness is not sustained for a sufficient period.

The oospores of *P. cristata* were found to be associated with both the crop residues and seeds of oilseed poppy. However, no primary infections of poppy plants from either of these sources were detected throughout the course of this project. This indicates that, if it occurs, the rate of primary infections via either of these methods is very low. Low infection rates do not preclude either of these as methods of overwintering, as it has been shown that poppy downy mildew epidemics are able to develop from very low amounts of primary infections. However, the dominant method of overwintering appears to be on regrowth oilseed poppy and other poppy species. Regrowth poppies and other hosts of *P. cristata* such as *Pap. bracteatum, Pap. dubium, Pap. orientale, Pap. rhoeas* and *Pap. setigerum,* are found growing in headlands, on roadsides and gardens throughout the winter months. Climatic conditions in Tasmania during the winter months appear to be favourable for conidium infection and conducive to the overwintering of *P. cristata* on these hosts. Weather monitoring during the winter of 2002 predicted the occurrence of both sporulation and infection. The ability of *Peronospora* spp. inoculum to be transported large distances in single seasons (Davis *et al.* 1985) means that survival of *P. cristata* is only required in one part of Tasmania, for the initiation of statewide epidemics each season.

The results from this project have highlighted a few key factors that should be considered for the control of *P. cristata* epidemics. The control of weed poppies, both regrowth oilseed poppy and other *Papaver* spp., during the winter months should be paramount to minimise the extent of overwintering by *P. cristata*, and thus reduce the levels of primary inoculum in subsequent seasons. As epidemics are primarily driven by secondary inoculum, once primary infection has occurred steps should be taken to reduce the generation of secondary inoculum. The reduction of the density of poppy crop canopies, either through sowing density or the development of cultivars with reduced leaf area, could be considered to reduce relative humidity levels of the crop microclimate. The timing of chemical fungicide applications to coincide with infection events within the crop based on weather conditions, rather than the current calendar based regimes, could also be used to increase the effectiveness of application, and potentially reduce application frequency. To this end, the use of a forecaster model, such as the modified form of DOWNCAST outlined in this thesis, would be beneficial.

7.2. Suggestions for further research

The current ambiguities in the host ranges of the species *P. arborescens* and *P. cristata* need to be addressed. The results of this project call into question the status of *P. arborescens* as a pathogen of oilseed poppy, as molecular evidence has indicated that these are in fact two separate species, and that *P. cristata* is the pathogen of oilseed poppy in Tasmania. The host range study employed in this project was of only limited scope and should not be considered definitive. If *P. arborescens* is not a pathogen of oilseed poppy then previous research into the pathogen of oilseed poppy should be attributed to *P. cristata*. If *P. arborescens* is a

pathogen of oilseed poppy, difficulty may exist in determining the species present in previous studies. In addition, the question of the relative pathogenicities of the two species to oilseed poppy is of some importance. Whether or not *P. arborescens* is present in Australia also needs to be answered. If *P. arborescens* isn't present in Australia the prevention of its introduction by quarantine measures may be an issue.

This project has shown that the alkaloid content of poppy capsules is reduced by downy mildew infection. However, the effect of downy mildew infection on the dry matter yield of poppy crops could not be distinguished. Therefore a dedicated crop yield components study assessing the effect of downy mildew infection on dry matter yield and alkaloid content on individual plants is necessary to determine if dry matter yield is affected by downy mildew infection.

Further validation of the predictive model DOWNCAST is required. Due to time constraints on this project and the relatively short growing season of oilseed poppy in Tasmania, only limited validation could be conducted. Several more season's worth of data are required to confirm DOWNCAST as an accurate predictor of poppy downy mildew epidemics. Investigation of the quantifying forecasting models, ONIMIL and ZWIPERO, as alternatives to DOWNCAST should be considered. The recently described ZWIPERO especially, may be preferable to DOWNCAST as predictions by ZWIPERO are based on the relative favourability of conditions to epidemics rather than the stringent critical limits that are the basis of prediction by DOWNCAST. Under borderline conditions for epidemic development, the occurrence of low rates of infection may not be predicted by DOWNCAST, but may be accounted for in the relative scaling of ZWIPERO. As part of this validation, more detailed laboratory studies on the influence of environmental conditions are

necessary. The studies of the influence of temperature and relative humidity on sporulation by *P. cristata* conducted in this project had only limited replication and were designed to quickly confirm, or deny, the most important parameters used in the sporulation sub-unit of DOWNCAST. Amongst these investigations should be a study into the perceived interaction between the critical limit of relative humidity for sporulation, and ambient temperature.

Oospores were associated with both the residues and seed of oilseed poppy crops, however no evidence of primary infection from either of these sources was detected. Therefore the ability of the oospores of *P. cristata* to germinate needs to be investigated. Previous laboratory studies have reported difficulty in inducing the germination of oospores of the downy mildew pathogen (Behr 1956; Kothari & Prasad 1970). If oospore germination does occur, then investigations into the infection of oilseed poppy by oospores should be undertaken, especially to determine if pre-emergence infection of poppy leads to seedling death prior to emergence.

Analysis of weather data collected during the winter of 2002 indicated that in the presence of viable plant hosts, *P. cristata* was capable of overwintering through conidium production alone. To confirm that the winter climate of Tasmania is favourable for overwintering by this method, historical weather data could be analysed by DOWNCAST or an alternative predictive model. Such a study could be part of an analysis of regional climatic data to provide a regional risk assessment for poppy downy mildew. However, for this to be feasible a source of leaf wetness data, or a means of calculating leaf wetness based on measured variables, is required. If compatible historical data cannot be obtained, then regional weather monitoring over several years could provide an alternative.

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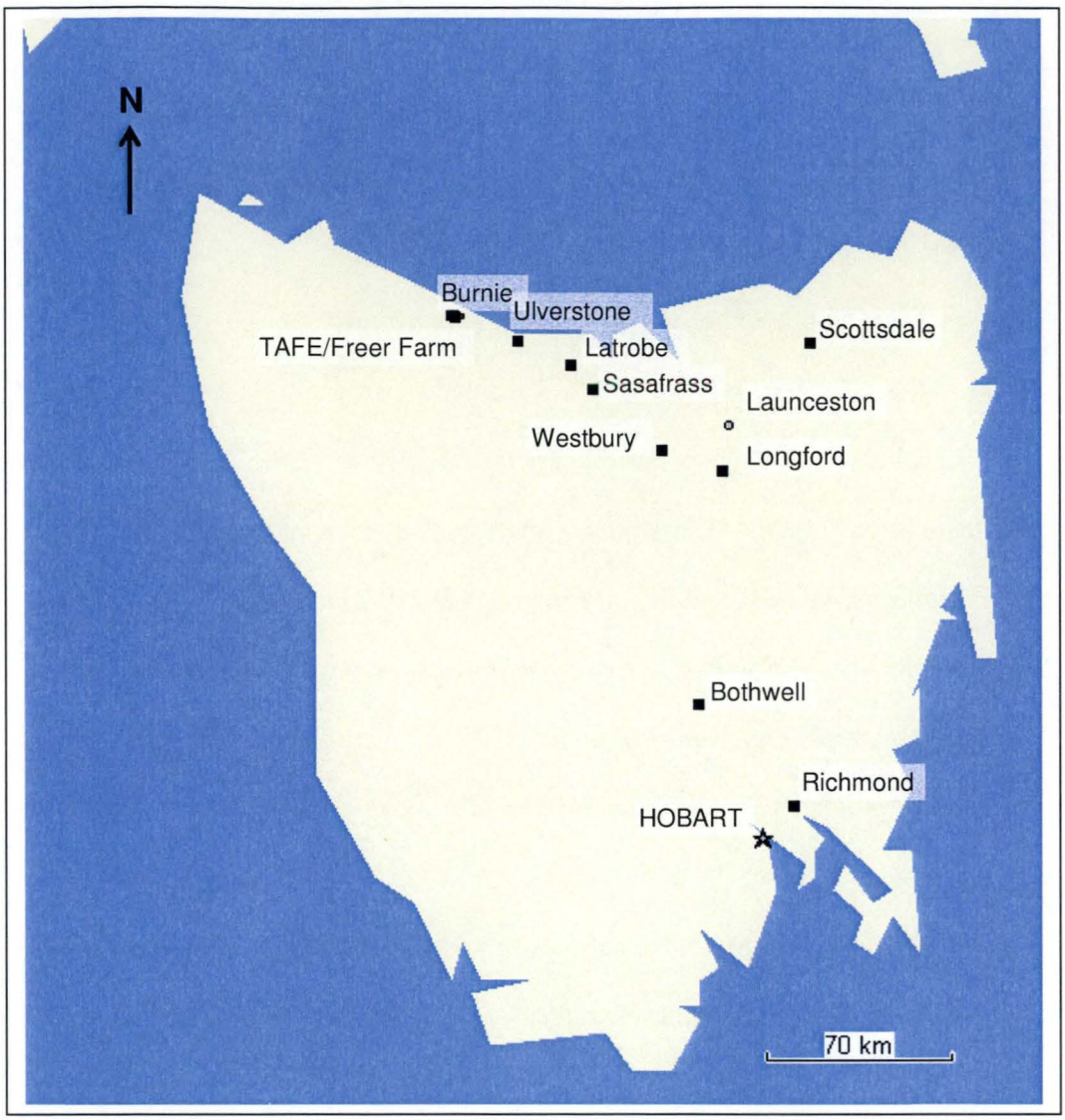
Appendix I: Map of Tasmania



Appendix II: Previous publications from this project

Symposium paper:

Scott, J.B., Hay, F.S., Wilson, C.R., Cotterill, P.J., and Fist, A.J. (2003). Spatiotemporal analysis of epiphytotics of downy milew of oilseed poppy in Tasmania, Australia. *Phytopathology* **93**: 752-757

(see reprint attached)

Conference poster:

Scott, J. B., Hay, F. S., Wilson, C. R., Cotterill, P. J. and Fist, A. J. (2001). Epidemiology of downy mildew of oilseed poppy in Tasmania. *13th Biennial Plant Pathology Conference*. Australasian Plant Pathology Society. Cairns. 24-27 Sept. 2001

Appendix III: Effect of plant and leaf age on infection susceptibility of *Papaver somniferum* to *Peronospora cristata*

Introduction

A short pilot study was conducted to aid the determination of what age leaves to sample for disease monitoring during field studies, and what age seedlings would be optimal for disease monitoring. This study looked at the influence of plant and leaf age on infection by *Peronospora cristata.*

Materials and methods

Oilseed poppy seedlings were grown from surface sterilised seed sown into 20 cm diameter pots 4/5th filled with potting mix (Appendix V) and topped with seed raising mix (Appendix V). Seeds were surface sterilised by immersion for five minutes in bleach (0.5 % available chlorine), followed by two rinses in SDW for five minutes each. Pots were then maintained under glasshouse conditions (23±0.1/15±0.1 °C and 73.4±0.4/87.0±0.3 % relative humidity, day/night, ±standard error of mean) until artificial infection. After germination seedlings were thinned to two per pot. The timing of sowing was staggered to produce seedlings of varying developmental ages at the same time for further work.

Ten plants each of four developmental ages, eight leaf, large rosette, bud in apex and hook, were misted with conidium suspension to induce infection. Conidium suspensions were generated by incubating downy mildew infected leaves harvested from culture in sealed plastic containers with moist tissues, at 12 °C and 100 % relative humidity for 12 hours under dark conditions. Conidia were collected by

shaking leaves in distilled water and the suspension was adjusted to 10^4 conidia.mL^{-1} with the aid of a haemocytometer. Plants were misted to the point of run off and then incubated in a misting chamber overnight under glasshouse conditions. Leaf wetness was maintained by regular fine mists, applied for 10 s at five minute intervals during daylight hours, and 30 min intervals under dark conditions. Following incubation plants were maintained for 14 days under dry glasshouse conditions (22±0.1/14±0.1 °C, 64±0.6/79±0.4 % relative humidity, day/night, ±standard error of mean), with basal watering to maintain low relative humidity. An additional two plants of each developmental age were also maintained under dry glasshouse conditions as a negative control. At the end of the 14 day period every leaf of every plant was scored for the both the incidence and severity of downy mildew infection. Leaf age was determined by counting the leaf number from the base of the plant, with the eldest leaf labeled 'leaf 1'. Downy mildew severity was determined as the percentage of total leaf area, with the aid of disease severity diagrams (Appendix IV).

Results

The highest proportion of plants infected by downy mildew (0.9) was observed from the eight leaf and large rosette developmental ages, whilst the lowest incidence (0.5) occurred at hook (Table IIIA). No significant difference was observed in the incidence of leaf infection among plant developmental ages, with the highest incidence (0.162) observed at plant age 2 (Table IIIA). The highest severity of infection occurred at large rosette (0.759), whilst the lowest (0.221) occurred at hook stage (Table IIIA). No infection was detected in any negative controls.

Table IIIA Susceptibility of oilseed poppy to infection by *Peronospora cristata* as a function of plant age.

Plant age[1]	Plant infection incidence[2]	Leaf infection incidence[3]	Leaf infection severity (%)[4]
Eight leaf	0.90	0.133	0.500
Large rosette	0.90	0.162	0.759
Bud in apex	0.70	0.148	0.478
Hook	0.50	0.095	0.221
s.e.m.[5]	0.25	0.098	0.271

[1]developmental age of plant at the time of infection

[2]incidence (as a proportion) of infection of individual plants

[3]incidence (as a proportion) of infection of individual leaves

[4]mean severity of leaf infection over all leaves (as a percentage of leaf area)

[5]standard error of the mean

Both the highest incidence and severity of leaf infection over all plant ages was observed at leaf 1, 0.425 and 1.94 % respectively (Table IIIB). No infections were observed of leaves 7 or 9 to 14.

Table IIIB Susceptibility of the leaves of oilseed poppy to infection by *Peronospora cristata* as a function of leaf age.

Leaf age[1]	# leaves[2]	Leaf infection incidence[3]	Leaf infection severity (%)[4]
1	40	0.425	1.94
2	40	0.325	1.33
3	40	0.300	1.01
4	40	0.175	0.48
5	40	0.125	0.36
6	40	0.025	0.03
7	40	0	0
8	40	0.050	0.05
9	37	0	0
10	26	0	0
11	15	0	0
12	10	0	0
13	4	0	0
14	2	0	0
s.e.m.[5]	-	0.221	0.45

[1]age of leaves present at the time of infection, with eldest living leaf classified as 1, second eldest living leaf classified as 2, and so on.

[2]total number of leaves of each age present for assessment

[3]incidence (as a proportion) of infection of individual leaves

[4]mean severity of leaf infection (as a percentage of leaf area)

[5]standard error of the mean

Discussion

The results of this study indicate that the plant development age most susceptible to infection by downy mildew was large rosette. Plants at large rosette at the time of infection were assessed as having the highest plant incidence, leaf incidence and severity of downy mildew infection. However, based on standard errors of the mean, little significant difference occurred between plants at large rosette and plants at

eight leaf at the time of infection. The eldest plants at the time of infection (hook) had the lowest plant incidence, leaf incidence and severity of downy mildew infection. These results indicate that the susceptibility of oilseed poppy plants to infection by downy mildew decreases with increasing plant age, which agrees with the observations of Behr (1956), and Kothar and Prasad (1970). Based on these results it was determined that the optimum plant age for use as trap plants for the monitoring of downy mildew epidemics, was small (or 10 leaf) to large rosette.

The eldest leaves plants had the highest incidence and severity of downy mildew infection. Both disease incidence and severity decreased with decreasing leaf age, with no leaf infections recorded for leaves of age 9 or greater. The greater suceptibility to downy mildew infection of older leaves agrees with the observations of Behr (1956), Nigram *et al.* (1989) and Yossifovitch (1929). The greater susceptibility of older leaves to infection by downy mildew may be related to the senescence of leaves. In a study of the susceptibility of pea leaves to infection by *P. viciae*, Mence and Pegg (1971), found that susceptibility decreased with age until leaves reach the beginning of senescence, at which point susceptibility increased. Another factor in the increased susceptibility of the eldest leaves may be their growth habit. The younger poppy leaves have a more erect growth habit than older leaves, which could promote moisture run off and reduce the build up of leaf wetness. The more prostrate older leaves would therefore be subjected to greater periods of leaf wetness, and therefore theoretically greater periods conducive to infection. Based on these results it was determined that leaves would be sampled from the bottom five nodes of poppy plants for the monitoring of disease incidence and severity.

Appendix IV: Disease severity key

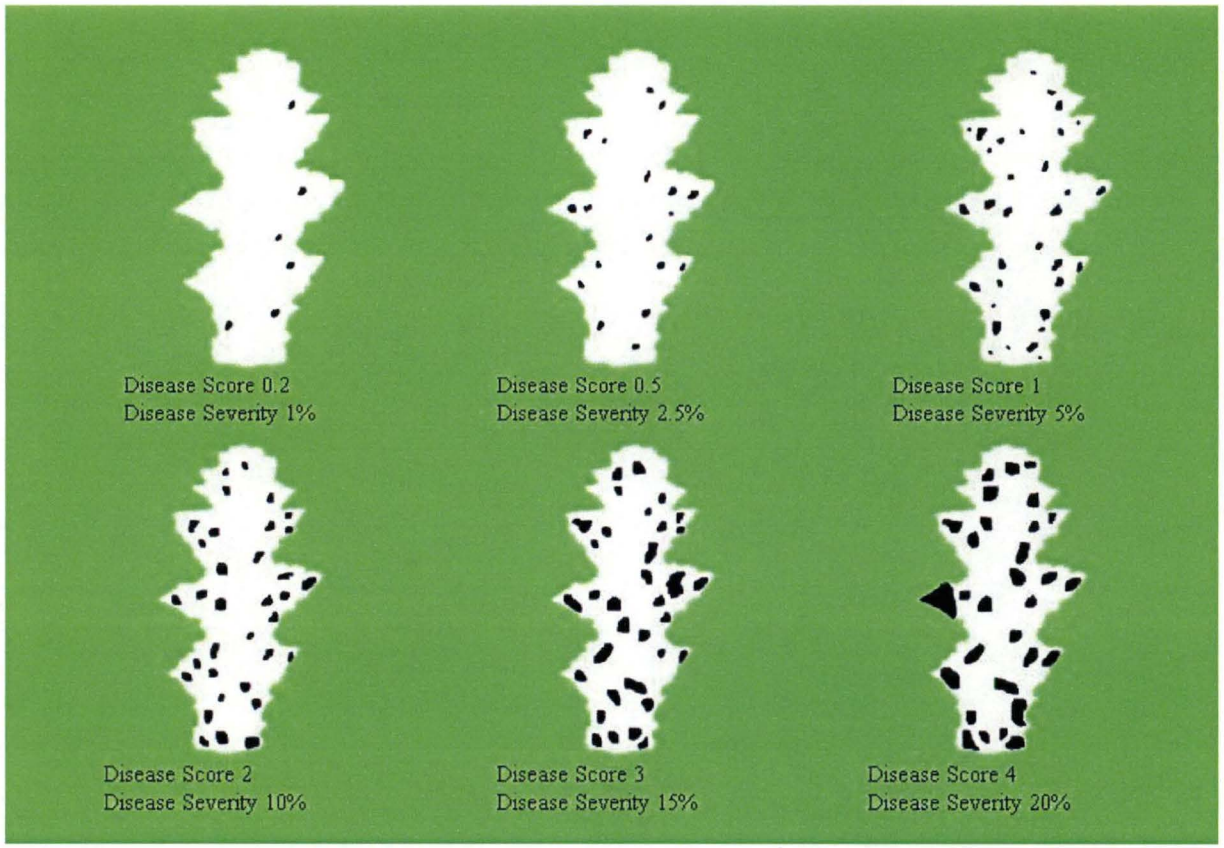
Disease severity was assessed using a severity key based on that designed for pea downy mildew (Falloon *et al.* 1995).

To create a diagrammatic representation of a poppy leaf, a single poppy leaf was collected from a glasshouse grown plant at the large rosette stage of development. This leaf was photocopied and scanned into a .jpg format. Using Microsoft® Paint (Version 4.0, Microsoft Corp.) the background of the scanned leaf was manually coloured aqua, whilst the leaf area was coloured white. This was used as a standard template for each individual diagram. Disease lesions were then manually drawn on the leaf area in red using Microsoft Paint.

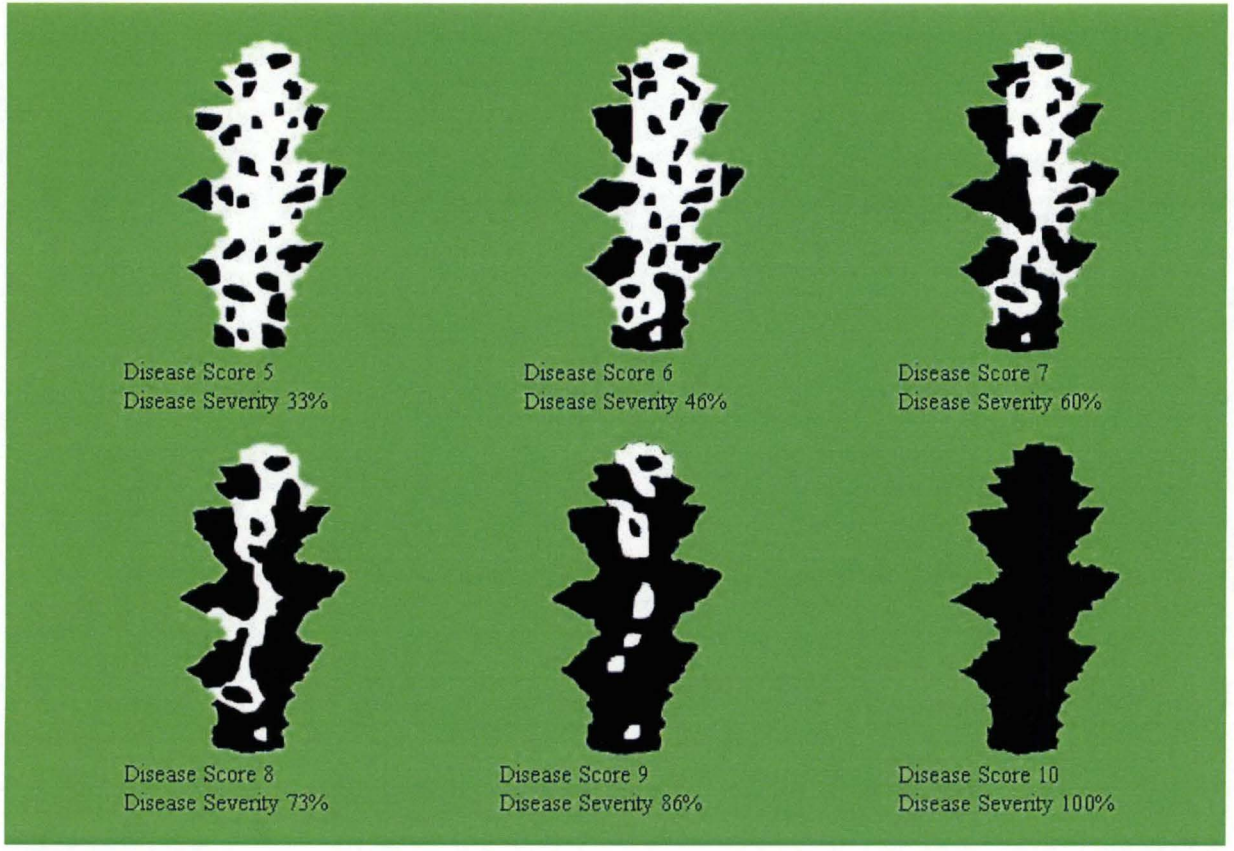
Scanned tagged image file format (.tif) images of the poppy leaf with areas of disease were imported into Imagine (Version 8.4®, ERDAS, Inc.) as an image (.img) file to measure leaf and disease areas. An unsupervised, iterative self-organising data analysis technique (Tou & Gonzalez 1974) was used to develop 10 spectral classes initialised from statistics. Using the raster attribute editor, a visual interpretation of the classification was undertaken. Each class was assigned a class name (leaf, disease, background) and a distinct class colour used (white, red, blue respectively) to define the classes. Pixel counts provided in the raster attribute editor were used in a simple percentage calculation to determine the percentage disease area of the leaf:

$$ds = \frac{dp}{tp} \times 100$$

where *ds* is the disease severity as a percentage of leaf area, *dp* is the sum of diseased pixels, and *tp* is the sum of all pixels. Changes to the image file were saved and the file exported as tagged image file format files. Necessary corrections to disease areas were done to the .jpg image in Microsoft Paint to increase or decrease the proportion of diseased leaf area as required, and re-analysed in Imagine.



Appendix IVA Disease key used to assess disease severity for downy mildew of poppy, based on the work of Falloon *et al.* (1995). Black areas represent disease lesions; white areas represent healthy leaf tissue. Mean disease scores (X) can be converted to mean disease severity (Y) using the formulae $Y = 5X$ (for $X \leq 4$) and $Y = 13.3X - 33$ (for $X > 4$)



Appendix IVA con't.

Appendix V: Potting mixtures

Potting mix:

in 1 m^3 potting mix:

- 50 % pine bark
- 30 % sand (4 mm diameter)
- 20 % *Spaghnum* sp. moss
- 1,000 g super phosphate
- 320 g potash
- 2,000 g Osmocote® plus (8-9 months)
  15:9:12, N:P:K, plus micronutrients
  Scotts Australia Pty. Ltd., N.S.W., Australia
- 1,000 g blood and bone
- 2,000 g lime
- 1,600 g dolomite
- 1,200 g SaturAid (wetting agent)
  active ingredient 15 % Propylene oxide-ethylene oxide block polymer
  Debco Pty. Ltd., VIC, Australia

Seed raising mix:

Seed Raising Mixture
(containing composted pine bark)
Debco Pty. Ltd.

Appendix VI: Relative humidity control

Atmospheric control of relative humidity was achieved using a modified form of the agar dish isopiestic equilibration method of Harris *et al.* (1970). Relative humidity within a sealed container at equilibrium is related to the water potential of the solid substrate within that container by the equation:

$$\psi = \frac{1000 \cdot R \cdot T}{W_A} \cdot \ln\left(\frac{p}{p_0}\right)$$

where ψ is the water potential of the substrate, R is the gas constant (8.3143 $J.K^{-1}.mole^{-1}$), T is the temperature in Kelvin, W_A is the molecular weight of water (18.016), and p/p_0 is the relative humidity of the system as a proportion (Lang 1967). Once the required water potential is known for a given relative humidity at a set temperature, the required molality of solute can be calculated from the water potential using the equation:

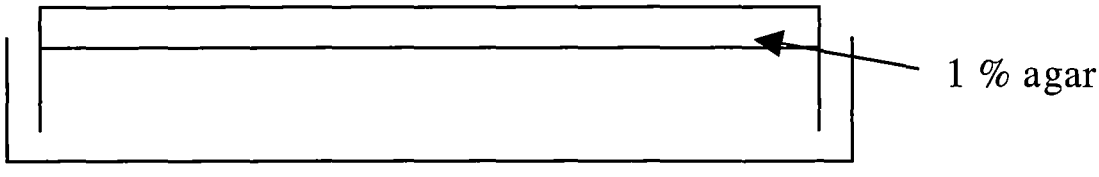
$$\psi = -\nu \cdot R \cdot T \cdot m \cdot \phi$$

where ν is the number of ions from one molecule of salt ($\nu = 2$ for sodium chloride), m is the molality (moles of solute per 1 kg of solvent) of the system, and ϕ is the osmotic coefficient (Lang 1967). The osmotic coefficient for a given temperature is calculated using the equation:

$$\phi = 1 - \frac{S_t \cdot \sqrt{d_0}}{A^3 \cdot m} \cdot \left[\left(1 + A \cdot \sqrt{m}\right) - 2 \cdot \ln\left(1 + A \cdot \sqrt{m}\right) - \frac{1}{1 + A \cdot \sqrt{m}} \right] + B \cdot m + C \cdot m^2 + D \cdot m^3$$

where S_t. $\sqrt{d_0}$, A, B, C and D are tabulated parameter values dependant on temperature (Lang 1967). Where values of S_t. $\sqrt{d_0}$, A, B, C and D were not available from Lang (1967) values were approximated by linear interpolation, as recommended by Lang (1967).

Water agar provides a good medium to act as the relative humidity regulating substrate, as the water potential of up to 2 % water agar is negligible (Harris *et al.* 1970). In this project varying concentrations of sodium chloride, depending on temperature and relative humidity requirements, was added to 1 % water agar (Powdered Agar Grade J3, Leiner Davis Gelatin (Australia), QLD, Australia). Amended agar was then poured into the base of petri dishes. Upon drying dishes were inverted (Appendix VIA) and sealed with plastic wrap.



Appendix VIA Diagrammatic representation of petri dishes used as controlled atmosphere containers.

Appendix VII: Weather data used to develop POPCAST prediction model

Appendix VIIA Field sporulation weather data collected from TAFE-Freer Farm, Burnie during 2001/2002 growing season.

Date (dd/mm)	Observation of sporulation[1]	Relative humidity								Mean night temp. (°C)	Total night rainfall (mm)	Preceding day temperatures			
		# hours continuous ≥				Total # hours ≥						# hours ≥			
		97 %	96 %	95 %	94 %	97 %	96 %	95 %	94 %			27 °C	28 °C	29 °C	30 °C
17/11	0	3	3	3	3	3	3	3	3	12.2	0.00	0	0	0	0
18/11	0	9	9	10	10	9	9	10	10	9.8	3.81	0	0	0	0
19/11	0	0	0	0	0	0	0	0	0	4.1	0.00	0	0	0	0
20/11	0	0	0	0	0	0	0	0	0	8.9	0.00	0	0	0	0
21/11	0	1	1	2	4	2	2	4	7	11.6	0.25	0	0	0	0
22/11	0	0	0	0	0	0	0	0	0	12.0	0.00	0	0	0	0
23/11	0	0	0	0	0	0	0	0	0	11.7	0.00	0	0	0	0
24/11	1	10	10	10	10	10	10	10	10	13.7	1.51	0	0	0	0
25/11	1	8	9	9	9	8	9	9	9	13.4	0.25	6	2	0	0
26/11	0	10	10	10	10	10	10	10	10	10.5	0.25	0	0	0	0
27/11	1	9	9	9	9	9	9	9	9	9.2	0.00	3	2	0	0
28/11	0	2	2	5	6	2	4	5	6	10	0.00	7	7	7	7
29/11	0	6	6	7	7	6	6	7	7	10.1	0.00	6	5	3	3
30/11	0	6	7	7	8	6	7	7	8	10.3	0.00	0	0	0	0
1/12	1	6	6	7	7	6	6	7	7	7.4	0.00	3	2	0	0
2/12	0	3	3	4	4	3	3	4	4	7.5	0.00	0	0	0	0
3/12	1	4	9	9	9	7	9	9	9	10.1	0.00	0	0	0	0
4/12	0	0	0	0	0	0	0	0	0	7.5	0.00	0	0	0	0
5/12	0	1	1	1	1	1	1	1	1	7.7	0.00	1	1	0	0
6/12	0	0	0	0	0	0	0	0	0	11.2	0.00	0	0	0	0
7/12	0	6	6	6	10	6	7	8	10	7.8	0.00	0	0	0	0

Appendix VIIA con't.

Date (dd/mm)	Observationof sporulation	Relative humidity								Mean night temp. (°C)	Total night rainfall (mm)	Preceding daytime temperatures			
		# hours continuous ≥				Total # hours ≥						# hours ≥			
		97 %	96 %	95 %	94 %	97 %	96 %	95 %	94 %			27 °C	28 °C	29 °C	30 °C
8/12	1	6	6	6	6	6	6	6	7	9.6	0.00	0	0	0	0
9/12	1	9	9	9	9	9	9	9	9	9.7	0.00	0	0	0	0
10/12	1	7	7	7	7	7	7	7	7	7.9	0.00	0	0	0	0
11/12	1	3	6	7	9	3	6	7	9	6.1	0.00	0	0	0	0
12/12	1	7	7	7	7	7	7	7	7	6.8	0.00	0	0	0	0
13/12	1	0	0	0	2	0	0	0	2	10.2	0.00	5	5	4	4
14/12	0	3	3	4	6	3	3	4	6	9.0	0.00	6	5	5	4
15/12	1	4	4	5	5	4	4	5	5	8.6	0.00	4	2	1	1
16/12	1	0	0	0	0	0	0	0	0	9.7	0.00	6	5	4	4

[1]score of '1' indicates sporulation, '0' indicates no sporulation

Appendix VIIB Field sporulation weather data collected from crop 1 at the TAFE-Freer Farm, Burnie during 2002/2003 growing season.

Date (dd/mm)	Observationof sporulation[1]	Relative humidity								Mean night temp. (°C)	Total night rainfall (mm)	Preceding day temperatures			
		# hours continuous ≥				Total # hours ≥						# hours ≥			
		97 %	96 %	95 %	94 %	97 %	96 %	95 %	94 %			27 °C	28 °C	29 °C	30 °C
10/12	0	0	3	5	6	0	3	5	6	6.4	0.00	0	0	0	0
11/12	1	5	6	6	6	5	6	6	6	10.4	0.00	0	0	0	0
12/12	1	1	1	3	3	1	1	3	3	10.6	0.00	0	0	0	0
13/12	0	0	0	0	0	0	0	0	0	10.4	0.00	0	0	0	0
14/12	0	2	2	3	4	2	2	3	5	9.0	0.00	0	0	0	0
15/12	1	4	7	7	7	4	7	7	7	12.1	0.00	0	0	0	0
16/12	0	1	4	4	4	2	4	4	4	12.2	0.00	0	0	0	0
17/12	0	0	2	3	6	0	3	5	6	13.9	0.00	0	0	0	0
18/12	1	7	7	7	8	7	7	7	8	11.6	0.00	3	0	0	0
19/12	1	9	9	10	10	9	9	10	10	14.5	0.00	0	0	0	0
20/12	1	6	7	7	7	6	7	7	7	14.3	0.00	0	0	0	0
21/12	1	6	7	7	7	6	7	7	7	16.6	0.00	3	1	0	0
22/12	1	6	8	9	9	6	8	9	9	15.1	0.00	3	0	0	0
23/12	0	0	0	0	0	0	0	0	0	8.4	0.00	0	0	0	0

[1]score of '1' indicates sporulation, '0' indicates no sporulation

Appendix VIIC Field sporulation weather data collected from crop 2 at the TAFE-Freer Farm, Burnie during 2002/2003 growing season.

Date (dd/mm)	Observationof sporulation[1]	Relative humidity # hours continuous ≥				Relative humidity Total # hours ≥				Mean night temp. (°C)	Total night rainfall (mm)	Preceding day temperatures # hours ≥			
		97 %	96 %	95 %	94 %	97 %	96 %	95 %	94 %			27 °C	28 °C	29 °C	30 °C
3/12	1	7	8	8	9	7	8	8	9	14.3	0.00	0	0	0	0
4/12	1	4	4	4	5	4	4	4	5	9.5	0.00	1	0	0	0
5/12	1	10	10	10	10	10	10	10	10	8.6	1.25	0	0	0	0
6/12	1	3	8	8	9	4	8	8	9	5.3	0.00	0	0	0	0
7/12	1	4	6	6	6	4	6	6	6	7.0	0.00	0	0	0	0
8/12	1	6	6	6	7	6	6	6	7	9.7	0.00	0	0	0	0
9/12	1	6	7	7	7	6	7	7	7	5.5	0.00	0	0	0	0
10/12	1	0	0	0	0	0	0	0	0	7.1	0.00	0	0	0	0
11/12	1	5	5	6	6	5	5	6	6	10.4	0.00	0	0	0	0
12/12	1	1	1	4	4	1	1	4	4	10.5	0.00	0	0	0	0
13/12	0	0	0	0	0	0	0	0	0	10.3	0.00	0	0	0	0
14/12	0	2	3	3	4	2	3	3	4	9.2	0.00	0	0	0	0
15/12	1	6	7	7	8	6	7	7	8	12.2	0.00	3	2	1	0
16/12	1	5	8	9	9	6	7	9	9	11.7	2.80	3	2	0	0
17/12	1	7	8	8	8	7	8	8	8	13.7	0.00	1	0	0	0
18/12	1	7	7	8	8	7	8	8	8	11.7	0.00	2	0	0	0
19/12	1	6	7	7	8	6	7	7	8	15.1	0.00	3	3	1	0
20/12	1	6	7	8	8	6	7	8	8	14.2	0.00	3	3	2	0
21/12	-[2]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22/12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23/12	0	0	0	0	0	0	0	0	0	8.3	0.00	0	0	0	0

[1]score of '1' indicates sporulation, '0' indicates no sporulation

[2]'-' indicates data lost

Appendix VIID Infection observations from artificial inoculations followed by incubation at ambient conditions, and predictions based on weather data collected.

Start date (d/m/yy)	# observed infected plants per day[1]				Day of infection[2]	Predicted day of infection[3] Critical leaf wetness limit														Mean temp (°C)[4]
	day 1	day 2	day 3	day 4		1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	
1/12/01	2	0	0	3	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	8.9
5/12/01	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
11/12/01	4	4	4	4	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	7.8
16/12/01	3	3	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
20/12/01	2	3	4	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15. 5
7/3/02	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
8/4/02	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	-
22/4/02	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
6/5/02	3	4	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	10.2
20/5/02	3	4	3	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
3/6/02	4	4	4	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	10.4
17/6/02	3	2	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2.0
1/10/02	3	4	3	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	12.8
14/10/02	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	-
29/10/02	1	3	1	3	2	0	0	0	0	0	0	2	2	2	2	2	2	2	2	14.1
14/11/02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
18/11/02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
23/11/02	2	3	1	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	17.1
3/12/02	2	3	0	2	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	11.5
10/12/02	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-

[1]observed number of infected plants (out of four) for groups of plants recovered after 1, 2, 3 and 4 days exposure to ambient conditions

[2]the first day of a period where more than one plant was infected was deemed the day of infection

[3]assuming a linear increase in leaf wetness values from 1.0 to critical leaf wetness limit over five hour period (DEW1), followed by three hours of leaf wetness greater than critical leaf wetness limit (DEW2), and critical limit indicating conidium death due to slow dew deposition (WETMORT) equal to DEW1

[4]mean temperature for infections predicted using a critical leaf wetness limit of 4.5, '-' indicates no infection.

Appendix VIII: Weather data collected during the monitoring of overwintering by the downy mildew pathogen

Appendix VIIIA Weather data collected during the monitoring of *Peronospora cristata* survival during the WINTER period of 2002 on potted *Papaver somniferum* plants.

Date	Temperature (°C)			Relative humidity (%)			Rainfall	Leaf wetness	
(d/mm/yy)	min.	mean	max.	min.	mean	max.	(mm)[1]	period[2]	total[3]
21/05/02	3.6	10.7	17.9	49.9	77.9	100.0	0.00	2	2
22/05/02	7.3	12.4	20.2	36.6	69.6	84.8	0.00	2	3
23/05/02	-0.3	7.3	18.7	38.5	80.3	100.0	0.75	6	10
24/05/02	0.1	6.9	19.0	45.6	84.4	100.0	9.15	11	17
25/05/02	-0.3	6.3	19.4	47.7	86.5	100.0	0.76	12	18
26/05/02	-0.8	7.0	18.7	55.0	89.2	100.0	0.50	12	18
27/05/02	1.0	8.0	19.0	48.6	84.1	100.0	1.02	11	14
28/05/02	1.4	8.2	19.0	44.1	82.7	100.0	0.50	6	8
29/05/02	0.6	7.9	18.7	45.8	86.9	100.0	5.33	11	18
30/05/02	-0.8	6.6	17.5	62.6	92.2	100.0	0.76	12	18
31/05/02	2.7	8.7	17.1	53.7	89.4	100.0	1.27	12	18
1/06/02	1.4	8.5	17.5	51.7	84.4	100.0	0.50	6	9
2/06/02	2.7	9.2	18.3	66.4	92.7	100.0	0.51	10	16
3/06/02	6.5	10.7	15.2	73.3	93.6	100.0	0.51	12	17
4/06/02	6.9	10.5	16.8	73.3	95.0	100.0	0.76	6	9
5/06/02	5.7	10.6	17.9	74.9	95.4	100.0	1.76	12	19
6/06/02	5.7	10.5	14.4	82.6	94.7	100.0	1.27	11	15
7/06/02	9.4	13.4	20.2	64.6	88.5	100.0	2.04	4	11
8/06/02	4.9	7.3	12.9	76.4	95.9	100.0	3.79	16	22
9/06/02	4.9	9.2	13.7	85.7	97.1	100.0	1.00	12	18
10/06/02	6.1	9.4	12.1	71.4	92.2	99.5	3.03	5	12
11/06/02	6.9	10.4	15.2	80.0	94.3	100.0	0.50	7	12
12/06/02	5.7	10.0	12.5	95.4	99.3	100.0	14.73	20	21
13/06/02	3.6	7.2	13.3	75.1	95.9	100.0	3.04	15	23
14/06/02	2.3	8.0	15.6	70.5	95.0	100.0	3.03	12	21
15/06/02	5.7	9.5	16.4	78.9	96.6	100.0	14.45	13	19
16/06/02	1.4	7.6	17.5	60.5	90.6	100.0	0.75	12	18
17/06/02	-1.3	5.2	15.2	49.4	87.8	100.0	0.50	12	18
18/06/02	-3.1	2.8	14.4	54.5	90.0	99.5	0.25	6	10
19/06/02	-1.3	6.2	11.9	75.8	93.0	100.0	0.50	7	16
20/06/02	6.3	9.7	12.1	78.9	90.0	100.0	3.55	9	13
21/06/02	5.3	8.8	14.1	74.6	93.9	100.0	1.26	4	11
22/06/02	8.6	10.5	13.9	68.7	86.2	97.9	0.00	4	4
23/06/02	6.1	10.0	15.0	69.3	93.6	100.0	0.25	8	11
24/06/02	10.5	11.9	13.9	85.8	95.9	100.0	6.60	11	21
25/06/02	7.5	10.1	12.5	81.4	91.7	99.8	6.57	15	17
26/06/02	1.7	7.2	11.7	77.2	92.1	100.0	0.50	6	11

Appendix VIIIA con't.

Date	Temperature (°C)			Relative humidity (%)			Rainfall	Leaf wetness	
(d/mm/yy)	min.	mean	max.	min.	mean	max.	(mm)[1]	period[2]	total[3]
27/06/02	3.0	8.1	10.9	81.0	93.6	100.0	8.61	15	18
28/06/02	0.6	6.0	13.9	54.6	85.5	100.0	0.25	7	9
29/06/02	1.7	6.9	14.5	60.0	86.9	100.0	0.00	2	3
30/06/02	1.9	6.6	11.1	81.6	94.9	100.0	0.00	6	9
1/07/02	4.7	8.9	15.2	65.9	92.0	100.0	0.25	7	15
2/07/02	3.2	8.2	12.3	73.2	95.2	100.0	8.36	11	20
3/07/02	3.0	6.5	10.7	95.5	99.5	100.0	3.51	24	24
4/07/02	2.8	6.4	11.1	74.9	94.5	100.0	2.26	12	15
5/07/02	5.7	8.4	11.7	72.6	92.1	100.0	10.41	11	14
6/07/02	3.4	6.3	10.5	73.4	92.4	100.0	1.25	13	13
7/07/02	6.1	9.3	13.5	65.6	85.7	97.5	0.00	1	1
8/07/02	9.6	11.4	14.1	84.8	93.6	97.9	0.75	10	20
9/07/02	6.3	8.9	11.3	79.7	95.2	100.0	6.75	20	20
10/07/02	3.8	7.7	14.1	62.8	86.7	99.5	4.00	0	0
11/07/02	3.6	7.5	10.9	66.1	87.7	100.0	0.25	5	6
12/07/02	5.9	10.0	13.9	85.2	95.2	99.5	0.00	24	24
13/07/02	7.7	10.2	13.3	87.8	96.8	100.0	0.00	24	24
14/07/02	5.3	9.0	12.7	64.3	91.8	100.0	0.00	12	15
15/07/02	5.7	9.6	12.7	96.2	98.7	100.0	0.25	17	23
16/07/02	4.5	9.5	11.9	88.0	96.5	100.0	3.00	24	24
17/07/02	1.4	5.8	11.7	68.6	92.3	100.0	1.75	15	15
18/07/02	4.9	10.5	16.1	70.0	93.6	98.7	0.00	2	2
19/07/02	11.3	11.9	13.3	88.4	93.5	97.3	0.00	2	3
20/07/02	4.9	8.7	12.1	73.7	93.9	100.0	0.50	10	11
21/07/02	3.2	7.1	11.5	73.8	90.2	100.0	0.00	1	1
22/07/02	6.5	8.5	10.5	86.6	96.7	100.0	0.00	9	12
23/07/02	7.0	9.9	13.3	86.3	97.9	100.0	0.00	17	21
24/07/02	1.3	5.9	12.0	55.1	84.6	100.0	0.00	3	3
25/07/02	-0.3	4.9	8.6	79.6	86.6	95.7	0.00	6	6
26/07/02	5.8	9.1	13.1	81.3	96.2	100.0	0.25	16	16
27/07/02	5.9	10.5	14.4	90.0	98.0	100.0	0.00	23	23
28/07/02	5.7	9.1	11.3	99.4	99.9	100.0	0.25	24	24
29/07/02	3.6	7.8	13.9	74.1	92.5	100.0	0.00	2	3
30/07/02	2.2	7.0	13.4	78.8	94.6	100.0	0.00	23	23
31/07/02	6.2	8.8	12.8	93.3	98.9	100.0	3.81	24	24
1/08/02	5.1	9.7	11.3	97.7	99.2	100.0	0.25	24	24
2/08/02	1.3	6.5	12.8	81.2	95.9	100.0	0.00	13	19
3/08/02	5.9	9.1	13.4	94.5	98.1	99.9	0.00	24	24
4/08/02	3.7	8.4	14.5	60.8	87.4	100.0	0.25	12	19
5/08/02	2.6	7.8	11.2	85.7	93.3	100.0	0.00	13	15
6/08/02	8.5	10.1	12.2	77.9	93.2	99.5	0.50	13	18
7/08/02	7.6	10.2	12.6	64.7	81.7	97.7	0.25	7	8
8/08/02	4.3	8.7	14.1	60.9	81.9	96.0	0.50	6	7
9/08/02	5.8	9.5	15.0	76.8	92.7	98.9	0.25	12	17
10/08/02	7.1	10.4	14.1	81.1	93.0	99.5	0.50	13	19

Appendix VIIIA con't.

Date	Temperature (°C)			Relative humidity (%)			Rainfall	Leaf wetness	
(d/mm/yy)	min.	mean	max.	min.	mean	max.	(mm)[1]	period[2]	total[3]
11/08/02	7.5	11.6	13.9	72.6	88.2	98.5	0.50	16	22
12/08/02	4.6	7.7	12.9	61.5	84.7	97.9	0.25	4	8
13/08/02	4.3	7.6	12.4	51.3	72.0	85.0	0.50	1	2
14/08/02	3.3	7.0	15.2	40.9	69.9	83.6	0.25	6	7
15/08/02	3.6	7.8	15.9	55.2	81.7	98.9	0.00	3	4
16/08/02	2.2	7.3	15.7	60.7	88.5	100.0	0.75	11	15
17/08/02	3.4	8.3	17.4	63.5	88.9	100.0	0.25	6	11
18/08/02	0.9	8.8	21.8	48.8	86.0	100.0	0.25	11	17
19/08/02	2.3	7.8	15.1	69.1	90.6	100.0	0.75	10	19
20/08/02	2.3	8.0	16.6	68.1	95.8	100.0	4.75	12	23
21/08/02	6.3	10.4	14.2	86.3	97.9	100.0	7.52	12	18
22/08/02	3.2	7.4	13.3	73.7	94.8	100.0	3.25	13	21
23/08/02	0.1	5.5	13.5	51.5	83.7	100.0	1.25	11	15
24/08/02	-1.2	6.6	19.0	44.4	79.4	99.6	0.00	6	8
25/08/02	0.6	8.4	22.4	42.5	80.8	99.6	0.25	6	11
26/08/02	0.1	7.9	20.0	48.9	86.6	100.0	0.25	12	18
27/08/02	2.5	8.8	19.2	57.6	88.6	100.0	0.00	11	17
28/08/02	5.6	11.9	22.5	52.1	87.1	100.0	0.25	11	17
29/08/02	8.6	10.7	11.7	99.5	100.0	100.0	12.51	24	24
30/08/02	5.4	10.0	14.5	73.0	93.4	100.0	17.12	12	18
31/08/02	4.0	9.6	18.8	71.0	93.3	100.0	1.75	11	19
1/09/02	1.1	8.8	19.6	66.4	90.6	100.0	1.76	10	16
2/09/02	2.6	9.7	15.6	89.5	98.5	100.0	3.75	11	21
3/09/02	10.0	11.5	14.3	73.4	93.1	100.0	10.00	14	22
4/09/02	4.6	8.9	13.4	65.0	91.8	99.8	9.75	12	21
5/09/02	5.8	10.4	14.0	83.6	94.9	99.5	1.25	11	17
6/09/02	4.9	10.2	15.3	74.1	93.2	100.0	3.25	10	18
7/09/02	3.4	7.4	12.5	74.8	92.6	100.0	4.75	12	23
8/09/02	3.1	7.9	12.3	85.7	96.7	100.0	5.50	11	21
9/09/02	0.5	6.4	10.4	69.2	86.9	96.7	6.50	10	20
10/09/02	-0.2	5.2	13.1	64.8	89.6	99.6	12.38	10	18
11/09/02	3.6	9.5	18.7	57.4	86.2	100.0	0.50	10	10
Mean	4.0	8.6	14.6	70.3	90.9	99.3	2.30	10.9	15.0

[1]total rainfall for the 24 hour period 0:01 to 24:00

[2]longest continuous period (in hours) of leaf wetness values $\geq$ 4.5 for the 24 hour period 0:01 to 24:00

[3]total number of hours of leaf wetness values $\geq$ 4.5 for the 24 hour period 0:01 to 24:00

Appendix VIIIB Weather data collected during the monitoring of *Peronospora cristata* survival during the MAY period of 2002 on potted *Papaver somniferum* plants.

Date	Temperature (°C)			Relative humidity (%)			Rainfall	Leaf wetness	
(d/mm/yy)	min.	mean	max.	min.	mean	max.	(mm)[1]	period[2]	total[3]
21/05/02	10.4	11.6	15.7	57.5	75.7	81.1	0.00	1	1
22/05/02	7.7	12.3	20.0	40.2	67.0	81.5	0.00	2	4
23/05/02	-2.6	6.3	18.8	35.1	75.6	98.3	0.00	6	7
24/05/02	-1.6	6.1	18.8	41.3	79.2	98.7	0.75	11	15
25/05/02	-2.7	4.5	18.7	44.1	83.8	100.0	0.25	4	6
26/05/02	-2.7	6.1	17.4	52.6	87.2	100.0	0.50	6	9
27/05/02	0.2	8.2	17.9	45.4	79.2	99.6	0.75	11	13
28/05/02	-1.2	7.9	19.2	38.6	76.7	99.5	0.50	3	7
29/05/02	-1.4	7.3	18.9	41.4	83.5	100.0	5.59	7	16
30/05/02	-3.0	5.3	18.7	51.9	89.3	100.0	0.76	6	11
31/05/02	0.5	8.8	18.2	46.7	86.2	100.0	2.27	12	18
1/06/02	0.9	8.6	19.0	46.1	81.2	100.0	0.75	7	14
2/06/02	1.0	8.8	18.4	58.1	90.8	100.0	1.76	11	17
3/06/02	6.3	10.6	15.9	69.8	92.2	100.0	1.51	11	17
4/06/02	4.7	10.1	16.2	71.5	93.8	100.0	1.52	11	17
5/06/02	3.0	10.3	18.2	67.3	94.0	100.0	1.01	11	18
6/06/02	4.3	10.5	14.4	80.6	93.4	100.0	2.27	11	17
7/06/02	8.3	13.4	20.2	60.5	87.5	99.9	3.00	8	14
8/06/02	4.9	7.7	13.1	76.5	94.8	100.0	9.50	15	23
9/06/02	4.9	9.5	14.4	85.9	96.3	100.0	3.50	12	19
10/06/02	6.2	9.7	12.7	65.2	90.7	99.1	8.50	11	22
11/06/02	6.9	10.7	15.4	77.6	94.1	100.0	4.75	10	19
12/06/02	4.2	9.9	12.8	93.9	98.9	100.0	12.50	24	24
13/06/02	2.3	7.0	14.0	66.5	94.0	100.0	9.50	12	20
14/06/02	0.7	7.9	16.6	65.4	93.0	100.0	5.50	12	21
15/06/02	3.8	9.3	15.0	80.4	96.4	100.0	8.25	13	19
16/06/02	-0.3	7.0	19.1	52.4	88.0	100.0	5.00	11	17
17/06/02	-3.0	4.4	15.9	43.4	84.8	100.0	6.07	5	11
18/06/02	-5.0	2.4	16.2	40.0	85.3	100.0	0.00	2	4
Mean	2.0	8.3	16.9	58.5	87.3	98.5	3.32	9.2	14.5

[1]total rainfall for the 24 hour period 0:01 to 24:00

[2]longest continuous period (in hours) of leaf wetness values ≥ 4.5 for the 24 hour period 0:01 to 24:00

[3]total number of hours of leaf wetness values ≥ 4.5 for the 24 hour period 0:01 to 24:00

Appendix VIIIC Weather data collected during the monitoring of *Peronospora cristata* survival during the JUNE period of 2002 on potted *Papaver somniferum* plants.

Date	Temperature (°C)			Relative humidity (%)			Rainfall	Leaf wetness	
(d/mm/yy)	min.	mean	max.	min.	mean	max.	(mm)[1]	period[2]	total[3]
19/06/02	-3.6	6.1	12.5	76.4	92.3	100.0	1.01	5	11
20/06/02	5.3	9.6	13.5	70.6	89.8	100.0	7.59	9	15
21/06/02	3.8	8.8	14.7	69.6	93.1	100.0	3.29	11	17
22/06/02	8.2	10.5	15.0	64.7	86.1	98.3	1.77	6	7
23/06/02	4.8	10.0	15.8	67.1	92.9	100.0	2.28	11	19
24/06/02	10.5	12.0	14.8	83.7	94.6	100.0	10.92	9	18
25/06/02	7.3	10.3	13.5	81.3	90.0	99.5	11.68	18	18
26/06/02	-0.1	7.3	13.5	69.2	89.3	100.0	1.25	8	12
27/06/02	1.7	7.9	10.9	80.0	92.9	100.0	9.08	12	13
28/06/02	-1.7	6.2	14.1	47.8	79.4	100.0	5.75	6	10
29/06/02	-0.2	6.9	15.6	51.5	83.6	99.8	0.50	10	16
30/06/02	-0.1	6.6	12.5	71.7	92.3	100.0	1.00	12	18
1/07/02	2.3	9.1	17.9	57.1	88.0	100.0	1.75	11	17
2/07/02	0.8	8.1	14.3	62.1	92.0	100.0	3.75	11	20
3/07/02	0.8	5.8	12.1	83.8	97.7	100.0	5.50	15	23
4/07/02	1.3	6.4	13.5	63.0	90.0	100.0	5.00	11	17
5/07/02	4.3	8.6	13.9	62.6	88.1	99.5	4.75	11	18
6/07/02	2.1	6.3	12.1	59.5	88.7	100.0	3.75	13	17
7/07/02	5.3	9.5	15.2	56.5	81.9	95.8	3.50	6	9
8/07/02	9.4	11.5	14.8	78.7	90.2	95.5	4.00	10	20
9/07/02	6.3	9.1	12.1	76.5	90.9	98.7	3.75	12	18
10/07/02	2.3	7.5	16.8	51.6	82.8	99.1	2.75	6	8
11/07/02	1.5	7.3	12.5	60.8	83.9	99.8	2.50	10	15
12/07/02	4.1	10.5	17.6	62.7	87.3	97.9	3.00	12	18
13/07/02	5.7	10.4	15.8	70.1	90.7	99.5	2.50	11	17
14/07/02	4.0	9.4	16.2	54.1	86.6	99.5	2.00	12	18
15/07/02	3.8	9.3	14.3	86.8	96.2	100.0	2.25	14	20
16/07/02	2.3	9.4	13.9	78.3	92.3	99.1	2.25	11	18
17/07/02	-0.8	5.2	15.6	54.7	87.1	100.0	23.64	6	12
18/07/02	3.0	10.4	16.1	73.4	92.0	99.5	1.51	11	18
Mean	3.1	8.5	14.4	67.5	89.4	99.4	4.48	10.3	15.9

[1]total rainfall for the 24 hour period 0:01 to 24:00

[2]longest continuous period (in hours) of leaf wetness values ≥ 4.5 for the 24 hour period 0:01 to 24:00

[3]total number of hours of leaf wetness values ≥ 4.5 for the 24 hour period 0:01 to 24:00

Appendix VIIID Weather data collected during the monitoring of *Peronospora cristata* survival during the JULY period of 2002 on potted *Papaver somniferum* plants.

Date	Temperature (°C)			Relative humidity (%)			Rainfall	Leaf wetness	
(d/mm/yy)	min.	mean	max.	min.	mean	max.	(mm)[1]	period[2]	total[3]
19/07/02	11.2	12.0	14.0	84.5	92.4	97.7	0.00	4	7
20/07/02	2.6	9.0	13.7	60.6	89.8	100.0	3.75	11	17
21/07/02	0.7	7.3	13.8	64.4	87.5	100.0	3.25	11	12
22/07/02	5.8	8.5	11.4	83.1	96.1	100.0	1.25	11	18
23/07/02	4.6	9.8	15.3	73.0	96.3	100.0	1.00	14	20
24/07/02	-1.3	6.0	16.6	40.3	79.8	100.0	5.06	5	9
25/07/02	-3.6	4.8	10.2	61.1	81.7	98.4	0.00	1	2
26/07/02	3.5	9.1	15.5	65.2	93.8	100.0	14.40	12	18
27/07/02	2.8	10.5	17.8	75.9	94.2	100.0	1.75	11	17
28/07/02	3.7	8.5	11.3	97.7	99.8	100.0	9.00	24	24
29/07/02	1.0	7.8	18.2	61.3	89.6	100.0	6.75	11	17
30/07/02	-1.1	6.3	17.4	58.2	88.6	100.0	0.50	8	16
31/07/02	5.3	8.6	14.9	81.8	96.5	100.0	1.75	12	18
1/08/02	1.5	9.5	11.8	93.1	98.3	100.0	4.25	24	24
2/08/02	-1.6	6.2	18.4	51.0	88.1	100.0	3.02	6	11
3/08/02	3.2	9.4	17.2	71.4	92.8	100.0	5.82	10	18
4/08/02	0.0	8.5	18.9	41.7	83.1	100.0	0.50	10	16
5/08/02	-0.6	7.4	13.8	73.4	92.4	100.0	1.25	11	14
6/08/02	8.2	10.3	14.3	69.1	92.5	100.0	17.34	13	21
7/08/02	7.5	10.6	14.4	57.6	80.6	97.9	8.50	8	10
8/08/02	1.9	9.0	18.0	48.7	80.9	99.6	0.50	6	7
9/08/02	3.4	9.4	18.2	61.0	90.8	100.0	0.75	10	16
10/08/02	5.3	10.6	17.8	64.1	90.2	100.0	0.25	11	16
11/08/02	6.9	11.8	16.9	66.2	86.6	98.7	6.78	8	16
12/08/02	3.5	8.2	15.4	48.2	81.8	99.7	3.25	11	13
13/08/02	2.4	8.1	15.6	42.6	69.2	89.0	0.25	1	2
14/08/02	1.7	7.9	17.2	37.7	66.7	88.6	0.00	6	7
Mean	2.9	8.7	15.5	64.2	88.2	98.9	3.74	10.0	14.3

[1]total rainfall for the 24 hour period 0:01 to 24:00

[2]longest continuous period (in hours) of leaf wetness values $\geq$ 4.5 for the 24 hour period 0:01 to 24:00

[3]total number of hours of leaf wetness values $\geq$ 4.5 for the 24 hour period 0:01 to 24:00

Appendix VIIIE Weather data collected during the monitoring of *Peronospora cristata* survival during the AUGUST period of 2002 on potted *Papaver somniferum* plants.

Date	Temperature (°C)			Relative humidity (%)			Rainfall	Leaf wetness	
(d/mm/yy)	min.	mean	max.	min.	mean	max.	(mm)[1]	period[2]	total[3]
15/08/02	1.8	8.4	17.9	46.1	77.6	100.0	0.00	3	4
16/08/02	0.2	7.1	17.7	52.1	85.7	100.0	0.75	11	15
17/08/02	1.7	8.4	17.4	56.5	86.2	100.0	0.25	6	11
18/08/02	-0.7	8.5	19.7	51.0	84.4	100.0	0.25	11	17
19/08/02	0.7	7.5	16.8	62.8	88.2	100.0	0.75	9	19
20/08/02	0.7	7.7	16.6	69.8	94.2	100.0	4.75	12	23
21/08/02	6.3	10.6	15.0	80.7	95.3	100.0	7.52	12	18
22/08/02	3.1	7.8	16.9	64.7	90.1	100.0	3.25	13	21
23/08/02	-1.2	6.0	15.8	41.8	77.5	100.0	1.25	11	15
24/08/02	-2.1	6.0	16.3	44.6	75.7	97.7	0.00	6	8
25/08/02	-0.2	7.7	17.9	50.1	80.2	98.1	0.25	6	11
26/08/02	-1.3	7.5	20.2	51.0	83.2	100.0	0.25	12	18
27/08/02	1.4	8.4	19.0	61.6	85.3	100.0	0.00	11	17
28/08/02	5.8	12.0	20.9	55.0	83.7	99.5	0.25	11	17
29/08/02	8.4	10.7	11.7	94.1	97.4	99.6	12.51	24	24
30/08/02	5.0	10.7	17.2	57.9	86.0	100.0	17.12	12	18
31/08/02	2.8	9.3	16.4	66.9	89.4	100.0	1.75	11	19
1/09/02	-0.2	8.1	18.2	61.9	87.1	100.0	1.76	10	16
2/09/02	1.5	9.7	17.3	78.1	94.4	100.0	3.75	11	21
3/09/02	10.5	12.2	16.8	57.1	84.9	97.9	10.00	14	22
4/09/02	3.7	9.6	14.8	51.3	82.2	96.2	9.75	12	21
5/09/02	5.8	10.8	14.4	75.0	87.7	94.7	1.25	10	16
6/09/02	4.3	10.6	16.6	60.3	85.1	97.9	3.25	9	17
7/09/02	3.7	8.1	15.1	60.3	84.2	97.9	4.75	12	23
8/09/02	3.0	8.3	13.1	76.1	90.5	96.0	5.50	11	21
9/09/02	-1.0	7.0	11.8	57.0	78.2	93.3	6.50	10	20
10/09/02	-1.5	5.6	15.1	51.9	81.5	96.4	12.38	10	18
11/09/02	0.0	7.2	20.0	0.0	56.0	97.3	0.50	10	10
Mean	2.2	8.6	16.7	58.4	84.7	98.7	3.94	10.7	17.1

[1]total rainfall for the 24 hour period 0:01 to 24:00

[2]longest continuous period (in hours) of leaf wetness values ≥ 4.5 for the 24 hour period 0:01 to 24:00

[3]total number of hours of leaf wetness values ≥ 4.5 for the 24 hour period 0:01 to 24:00

Appendix VIIIF Weather data collected during the monitoring of *Peronospora cristata* sporulation on potted *Papaver somniferum* plants during June, 2002.

Date	Temperature (°C)			Relative humidity (%)			Rainfall	Leaf wetness	
(d/mm/yy)	min.	mean	max.	min.	mean	max.	(mm)[1]	period[2]	total[3]
3/06/02	6.6	10.7	14.6	74.0	92.9	100.0	0.00	2	2
4/06/02	6.2	10.2	14.4	78.7	95.6	100.0	0.00	3	5
5/06/02	4.8	10.3	15.7	78.5	95.9	100.0	2.79	12	19
6/06/02	5.1	10.6	14.2	81.3	93.3	100.0	0.76	10	13
7/06/02	8.1	13.0	16.2	77.8	89.7	100.0	2.54	2	4
8/06/02	5.3	7.5	10.6	86.6	96.5	100.0	4.54	9	21
9/06/02	5.9	9.4	13.1	90.5	97.2	100.0	0.75	8	15
10/06/02	6.9	9.6	11.4	74.3	92.0	99.5	5.57	5	10
11/06/02	7.4	10.5	13.5	86.8	94.9	100.0	2.25	10	13
12/06/02	8.8	10.9	12.9	94.1	98.9	100.0	15.98	9	16
Mean	6.5	10.3	13.7	82.2	94.7	100.0	3.52	7.0	11.8

[1]total rainfall for the 24 hour period 0:01 to 24:00

[2]longest continuous period (in hours) of leaf wetness values $\geq$ 4.5 for the 24 hour period 0:01 to 24:00

[3]total number of hours of leaf wetness values $\geq$ 4.5 for the 24 hour period 0:01 to 24:00